PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) **International Patent Classification 7 :**
A61K

A2

(11) **International Publication Number:** **WO 00/15178**

(43) **International Publication Date:** 23 March 2000 (23.03.00)

(21) **International Application Number:** PCT/US99/21001

(22) **International Filing Date:** 14 September 1999 (14.09.99)

(30) **Priority Data:**

60/100,224	14 September 1998 (14.09.98)	US
60/100,290	14 September 1998 (14.09.98)	US
60/109,232	18 November 1998 (18.11.98)	US
60/109,234	18 November 1998 (18.11.98)	US
09/320,069	26 May 1999 (26.05.99)	US
09/320,270	26 May 1999 (26.05.99)	US
09/320,371	26 May 1999 (26.05.99)	US
09/320,372	26 May 1999 (26.05.99)	US
09/320,544	26 May 1999 (26.05.99)	US
09/320,545	26 May 1999 (26.05.99)	US

(71) **Applicant** *(for all designated States except US)*: NAVICYTE, INC. [US/US]; 9880 Campus Point Drive, San Diego, CA 92121 (US).

(72) **Inventors; and**
(75) **Inventors/Applicants** *(for US only)*: GRASS, George, M. [US/US]; 1506 Juniper Mountain Road, P.O. Box 1242, Tahoe City, CA 96145 (US). LEESMAN, Glen, D. [US/US]; 186 Nighthawk Lane, Hamilton, MT 59840–9307 (US). NORRIS, Daniel, A. [US/US]; 3145 Cowley Way #130, San Diego, CA 92117 (US). SINKO, Patrick, J. [US/US]; 2 Country Place, Lebanon, NJ 08833 (US). WEHRLI, John, E. [US/US]; 4225 Corte de la Siena, San Diego, CA 92130 (US).

(74) **Agents:** POSTNER, Marya, A.; Cooley Godward LLP, 3000 El Camino Real, Five Palo Alto Square, Palo Alto, CA 94306–2155 (US) et al.

(81) **Designated States:** AU, CA, JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
Without international search report and to be republished upon receipt of that report.

(54) **Title:** PHARMACOKINETIC–BASED DRUG DESIGN TOOL AND METHOD

(57) **Abstract**

The present invention relates to a pharmacokinetic–based design and selection tool (PK tool) and methods for predicting absoption of an administered compound of interest. The methods utilize the tools, and optionally a separately operable component or subsystem thereof. The PK tool includes as computer–readable components: (1) input/output system; (2) physiologic–based simulation model of one or more segments of a mammalian system of interest having one or more physiological barriers to absorption that is based on the selected route of administration; and (3) simulation engine having a differential equation solver. The invention also provides methods for optimizing as well as enabling minimal input requirements a physiologic–based simulation model for predicting *in vivo* absorption, and optionally one or more additional properties, from either *in vitro* or *in vivo* data. The PK tool of the invention may be provided as a computer system, as an article of manufacture in the form of a computer–readable medium, or a computer program product and the like. Subsystems and individual components of the PK tool also can be utilized and adapted in a variety of disparate applications for predicting the fate of an administered compound. The PK tool and methods of the invention can be used to screen and design compound libraries, select and design drugs, as well as predict drug efficacy in mammals from *in vitro* and/or *in vivo* data of one or more compounds of interest. The PK tool and methods of the invention also find use in selecting, designing, and preparing drug compounds, and multi–compound drugs and drug formulations (i.e., drug delivery system) for preparation of medicaments for use in treating mammalian disorders.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania
AM	Armenia
AT	Austria
AU	Australia
AZ	Azerbaijan
BA	Bosnia and Herzegovina
BB	Barbados
BE	Belgium
BF	Burkina Faso
BG	Bulgaria
BJ	Benin
BR	Brazil
BY	Belarus
CA	Canada
CF	Central African Republic
CG	Congo
CH	Switzerland
CI	Côte d'Ivoire
CM	Cameroon
CN	China
CU	Cuba
CZ	Czech Republic
DE	Germany
DK	Denmark
EE	Estonia
ES	Spain
FI	Finland
FR	France
GA	Gabon
GB	United Kingdom
GE	Georgia
GH	Ghana
GN	Guinea
GR	Greece
HU	Hungary
IE	Ireland
IL	Israel
IS	Iceland
IT	Italy
JP	Japan
KE	Kenya
KG	Kyrgyzstan
KP	Democratic People's Republic of Korea
KR	Republic of Korea
KZ	Kazakstan
LC	Saint Lucia
LI	Liechtenstein
LK	Sri Lanka
LR	Liberia
LS	Lesotho
LT	Lithuania
LU	Luxembourg
LV	Latvia
MC	Monaco
MD	Republic of Moldova
MG	Madagascar
MK	The former Yugoslav Republic of Macedonia
ML	Mali
MN	Mongolia
MR	Mauritania
MW	Malawi
MX	Mexico
NE	Niger
NL	Netherlands
NO	Norway
NZ	New Zealand
PL	Poland
PT	Portugal
RO	Romania
RU	Russian Federation
SD	Sudan
SE	Sweden
SG	Singapore
SI	Slovenia
SK	Slovakia
SN	Senegal
SZ	Swaziland
TD	Chad
TG	Togo
TJ	Tajikistan
TM	Turkmenistan
TR	Turkey
TT	Trinidad and Tobago
UA	Ukraine
UG	Uganda
US	United States of America
UZ	Uzbekistan
VN	Viet Nam
YU	Yugoslavia
ZW	Zimbabwe

PHARMACOKINETIC-BASED DRUG DESIGN TOOL AND METHOD

INTRODUCTION

Technical Field

The present invention relates to computer-implemented pharmacokinetic simulation models and drug design.

Background

A. Pharmacokinetic Modeling

Pharmacodynamics refers to the study of fundamental or molecular interactions between drug and body constituents, which through a subsequent series of events results in a pharmacological response. For most drugs the magnitude of a pharmacological effect depends on time-dependent concentration of drug at the site of action (e.g., target receptor-ligand/drug interaction). Factors that influence rates of delivery and disappearance of drug to or from the site of action over time include absorption, distribution, metabolism, and elimination. The study of factors that influence how drug concentration varies with time is the subject of pharmacokinetics.

In nearly all cases the site of drug action is located on the other side of a membrane from the site of drug administration. For example, an orally administered drug must be absorbed across a membrane barrier at some point or points along the gastrointestinal (GI) tract. Once the drug is absorbed, and thus passes a membrane barrier of the GI tract, it is transported through the portal vein to the liver and then eventually into systemic circulation (i.e., blood and lymph) for delivery to other body parts and tissues by blood flow. Thus how well a drug crosses membranes is of key importance in assessing the rate and extent of absorption and distribution of the drug throughout different body compartments and tissues. In essence, if an otherwise highly potent drug is administered extravascularly (e.g., oral) but is poorly absorbed (e.g., GI tract), a majority of the drug will be excreted or eliminated and thus cannot be distributed to the site of action.

The principle routes by which drugs disappear from the body are by elimination of unchanged drug or by metabolism of the drug to a pharmacologically active or inactive form(s) (i.e., metabolites). The metabolites in turn may be subject to further elimination or metabolism. Elimination of drugs occurs mainly via renal mechanisms into the urine and to some extent via mixing with bile salts for solubilization followed by excretion through the GI tract, exhaled through the lungs, or secreted through sweat or salivary glands etc. Metabolism for most drugs occurs primarily in the liver.

Each step of drug absorption, distribution, metabolism, and elimination can be described mathematically as a rate process. Most of these biochemical processes involve first order or pseudo-first order rate processes. In other words, the rate of reaction is proportional to drug concentration. For instance, pharmacokinetic data analysis is based on empirical observations after administering a known dose of drug and fitting of the data by either descriptive equations or mathematical (compartmental) models. This permits summarization of the experimental measures (plasma/blood level-time profile) and prediction under many experimental conditions. For example after rapid intravenous administration, drug levels often decline mono-exponentially (first-order elimination) with respect to time as described in Equation 1, where Cp(t) is drug concentration as a function of time, Cp(0) is initial drug concentration, and k is the associated rate constant that represents a combination of all factors that influence the drug decay process (e.g., absorption, distribution, metabolism, elimination).

$$Cp(t) = Cp(0)e^{-kt} \qquad \text{(Eq. 1)}$$

This example assumes the body is a single "well-mixed" compartment into which drug is administered and from which it also is eliminated (one-compartment open model). If equilibrium between drug in a central (blood) compartment and a (peripheral) tissue compartment(s) is not rapid, then more complex profiles (multi-exponential) and models (two- and three-compartment) are used. Mathematically, these "multi-compartment" models are described as the sum of equations, such as the sum of rate processes each calculated according to Equation 1 (i.e., linear pharmacokinetics).

Experimentally, Equation 1 is applied by first collecting time-concentration data from a subject that has been given a particular dose of a drug followed by plotting the data points on a logarithmic graph of time versus drug concentration to generate one type of time-concentration curve. The slope (k) and the y-intercept (C0) of the plotted "best-fit" curve is obtained and subsequently incorporated into Equation 1 (or sum of equations) to describe the drug's time course for additional subjects and dosing regimes.

When drug concentration throughout the body or a particular location is very high, saturation or nonlinear pharmacokinetics may be applicable. In this situation the capacity of a biochemical and/or physiological process to reduce drug concentration is saturated. Conventional Michaelis-Menten type equations are employed to describe the nonlinear nature of the system, which involve mixtures of zero-order (i.e., saturation:concentration independent) and first-order (i.e., non-saturation:concentration dependent) kinetics. Experimentally, data collection and plotting are similar to that of standard compartment models, with a notable exception being that the data curves are nonlinear. Using a time versus concentration graph to illustrate this point, at very high drug concentration the data line is linear because the drug is being eliminated at a maximal constant rate (i.e., zero-order process). The data line then begins to curve in an asymptotic fashion with time until the drug concentration drops to a point where the rate process becomes proportional to drug concentration (i.e., first-order process). For many drugs, nonlinear pharmacokinetics applies to events such as dissolution of the therapeutic ingredient from a drug formulation, as well as metabolism and elimination. Nonlinear pharmacokinetics also can be applied to toxicological events related to threshold dosing.

Classical one, two and three compartment models used in pharmacokinetics require *in vivo* blood data to describe time-concentration effects related to the drug decay process, i.e., blood data is relied on to provide values for equation parameters. For instance, while a model may work to describe the decay process for one drug, it is likely to work poorly for others unless blood profile data and associated rate process limitations are generated for each drug in question. Thus, such models are very poor for predicting the *in vivo* fate of diverse drug sets in the absence of blood data and the like derived from animal and/or human testing.

In contrast to the standard compartment models, physiological-based pharmacokinetic models are designed to integrate basic physiology and anatomy with drug distribution and disposition. Although a compartment approach also is used for physiological models, the compartments correspond to anatomic entities such as the GI tract, liver, lung etc., which are connected by blood flow. Physiological modeling also differs from standard compartment modeling in that a large body of physiological and physicochemical data usually is employed that is not drug-specific. However, as with standard compartment models the conventional physiological models lump rate processes together. Also, conventional physiological models typically fail to incorporate individual kinetic, mechanistic and physiological processes that control drug distribution and disposition in a particular anatomical entity, even though multiple rate processes are represented *in vivo*. Physiological models that ignore these and other important model parameters contain an underlying bias resulting in poor correlation and predictability across diverse data sets. Such deficiencies inevitably result in unacceptable levels of error when the model is used to describe or predict drug fate in animals or humans. The problem is amplified when the models are employed to extrapolate animal data to humans, and worse, when *in vitro* data is relied on for prediction in animals or humans.

For instance, the process of drug reaching the systemic circulation for most orally administered drugs can be broken down into two general steps: dissolution and absorption. Since endocytotic processes in the GI tract typically are not of high enough capacity to deliver therapeutic amounts of most drugs, the drugs must be solubilized prior to absorption. The process of dissolution is fairly well understood. However, the absorption process is treated as a "black box." Indeed, although bioavailability data is widely available for many drugs in multiple animal species and in humans, *in vitro* and or *in vivo* data generated from animal, tissue or cell culture permeability experiments cannot allow a direct prediction of drug absorption in humans, although such correlations are commonly used.

B. Computer Systems and Pharmacokinetic Modeling

Computers have been used in pharmacokinetics to bring about easy solutions to complex pharmacokinetic equations and modeling of pharmacokinetic processes. Other computer applications in pharmacokinetics include development of experimental study designs, statistical data treatment, data manipulation, graphical representation of data, projection of drug action, as well as preparation of written reports or documents.

Since pharmacokinetic models are described by systems of differential equations, virtually all computer systems and programming languages that enable development and implementation of mathematical models have been utilized to construct and run them. Graphics-oriented model development computer programs, due to their simplicity and ease of use, are typically used for designing multi-compartment linear and non-linear pharmacokinetic models. In essence, they allow a user to interactively draw compartments and then link and modify them with other iconic elements to develop integrated flow pathways using pre-defined symbols. The user assigns certain parameters and equations relating the parameters to the compartments and flow pathways, and then the model development program generates the differential equations and interpretable code to reflect the integrated system in a computer-readable format. The resulting model, when provided with input values for parameters corresponding to the underlying equations of the model, such as drug dose and the like can then be used to simulate the system under investigation.

While tools to develop and implement pharmacokinetic models exist and the scientific literature is replete with examples, pharmacokinetic models and computer systems developed to date have not permitted sufficient predictability of the pharmacokinetic fate of extravascularly administered drugs in a mammal from *in vitro* cell, tissue or compound structure-activity relationship (SAR/QSAR) data. A similar problem exists when attempting to predict absorption of a compound in one mammal (e.g., human) from data derived from a second mammal (e.g., dog). For example, existing pharmacokinetic models of oral absorption use several different approaches to predict oral absorption and fraction dose absorbed (Amidon *et al., Pharm. Res., (1988)* 5:651-654; Chiou, W.L., *Int. J. Clin. Pharmacol. Ther., (1994)* 32:474-482; Chiou, W.L., *Biopharm.Drug Dispos., (1995)* 16:71-75; Dressman *et al., J. Pharm. Sci.,* (1985) 74:588-589; Lennernas *et al., J Pharm. Pharmacol.,* (1997) 49:682-686; Levet-Trafit *et al., Life Sciences., (1996)* 58:PL359-63; Sinko *et al., Pharm. Res., (1991)* 8:979-988; and Soria *et al.,. Biopharm. Drug Dispos., (1996)* 17:817-818). Unfortunately, these models are flawed as they make mathematical assumptions that limit prediction to particular compounds, and the correlation function is sigmoidal in shape (i.e., high/steep slope). Therefore the predictive power of such models for compounds outside a relatively small group is very limited. This is particularly true for collections of compounds possessing variable ranges of dosing requirements and of permeability, solubility, dissolution rates and transport mechanism properties. Other drawbacks include use of drug-specific parameters and values in pharmacokinetic models from the outset of model development, which essentially limits the models to drug-specific predictions. These and other deficiencies also impair generation of rules that universally apply to drug disposition in a complex physiological system such as the GI tract.

Extravascular administration of drugs is the preferred route for physicians, patients, and drug developers alike due to lower product price, increased patient compliance, ease of administration. Current assessment of the bioavailability of extravascularly administered drugs and lead drug compounds, as well as bioavailability of intravascularly administered compounds relative to specialized barriers to absorption such as the blood brain barrier, is limited in large part to animal and human testing. The economic and medical consequences of problems with drug absorption and variable bioavailability are immense. Failing to identify promising or potentially problematic drug candidates during the discovery and pre-clinical stages of drug development is one of the most significant consequences of problems with drug bioavailability. Accordingly, there is a need to develop a comprehensive, physiologically-based pharmacokinetic model and computer system capable of predicting drug bioavailability and variability in humans that utilizes relatively straightforward input parameters. Furthermore, considering the urgent need to provide the medical community with new therapeutic alternatives and the current use of high throughput drug screening for selecting lead drug candidates, a comprehensive biopharmaceutical computer-based tool that employs a modeling approach for predicting bioavailability of compounds and compound formulations is needed.

Relevant Literature

Various publications review gastrointestinal anatomy and physiology including motility, secretion, absorption, and digestion, as well as gastrointestinal pharmacology and physiology in gastrointestinal diseased individuals (See, e.g., L. Johnson ed., *Physiology of the Gastrointestinal Tract*, Second edition, Vol. 2, Ravind Press (1987); Kutchai, *Gastrointestinal System, Part IV., Principles of Physiology*, Mosby Press (1996); and Sleisenger, *Gastrointestinal Disease*, 3rd edition, Saunders (1983)). Sharget *et al.* (*Physiological Factors Related to Drug Absorption*, Applied Biopharmaceutics and Pharmacokinetics (1993)) review pharmacokinetics and compartment modeling. Various pharmacokinetic models of oral drug absorption are disclosed in Grass, G. (*Advanced Drug Delivery Reviews* (1997) 23:199-219); Amidon et al., (*Pharm. Res.* (1988) 5:651-654); Chiou, W.L., (*Int. J. Clin. Pharmacol. Ther.*, (1994) 32:474-482); Chiou, W.L., (*Biopharm. Drug Dispos.*, (1995) 16:71-75); Dressman et al., (*J. Pharm. Sci.*, (1985) 74:588-589); Lennernas et al., (*J Pharm. Pharmacol.*, (1997) 49:682-686); Levet-Trafit et al., (*Life Sciences.*, (1996) 58:PL359-63); Sinko et al., (*Pharm. Res.*, (1991) 8:979-988); and Soria et al.,. (*Biopharm. Drug Dispos.*, (1996) 17:817-818)).

SUMMARY OF THE INVENTION

The present invention relates to a pharmacokinetic-based design and selection tool (PK tool) and methods for predicting absorption of a compound in a mammalian system of interest. The methods utilize the tool, and optionally a separately operable component or subsystem thereof.

The PK tool comprises as computer-readable components: (1) input/output system; (2) physiologic-based simulation model of one or more segments of a mammalian system of interest having one or more physiological barriers to absorption that is based on the selected route of administration; and (3) simulation engine having a differential equation solver, and optionally, a control statement module. The physiologic-based simulation model of the PK tool of the invention is a multi-compartment mathematical model comprising as operably linked components: (i) differential equations for one or more of fluid transit, fluid absorption, mass transit, mass dissolution, mass solubility, and mass absorption for one or more segments of the mammalian system of interest; and (ii) initial parameter values for the differential equations corresponding to physiological parameters and selectively optimized adjustment parameters, and optionally regional correlation parameters, for one or more segments of the mammalian system of interest; and, optionally, (iii) control statement rules for one or more of transit, absorption, permeability, solubility, dissolution, concentration, and mathematical error correction for one or more segments of the mammalian system of interest.

The computer-readable input/output system, physiologic-based simulation model, and simulation engine of the PK tool are capable of working together to carrying out the steps of: (1) receiving through the input/output system data comprising dose, permeability and solubility data of a compound of interest for one or more segments of the mammalian system of interest; and (2) applying the physiologic-based simulation model and simulation engine to generate an absorption profile for the compound characterized by one or more of concentration, rate of absorption, and extent of absorption relative to a selected sampling site that is across a physiological barrier for one or more segments of the mammalian system of interest.

The present invention also provides a database for utilization in the PK tool and method of the invention. The database includes one or more physiologic-based simulation models of the invention. Additional databases are provided for simulation model parameters, and may be integrated or separate from a database having a simulation model of the invention. The database(s) includes one or more of (i) differential equations for one or more of fluid transit, fluid absorption, mass transit, mass dissolution, mass solubility, and mass absorption for one or more segments of the mammalian system of interest; (ii) initial parameter values for the differential equations corresponding to physiological parameters and selectively optimized adjustment parameters, and optionally regional correlation parameters, for one or more segments of the mammalian system of interest; and (iii) control statement rules for one or more of transit, absorption, permeability, solubility, dissolution, concentration, and mathematical error correction for one or more segments of the mammalian system of interest. The database(s) have a compartment-flow data structure that is portable into and readable by a simulation engine for calculating time-dependent rate of absorption, extent of absorption, and concentration of a compound at a sampling site across a physiological barrier of one or more segments of the mammalian system of interest.

The invention also includes a method for selectively optimizing a pharmacokinetic-based simulation model for use in the PK tool of the invention. This method permits the PK tool of the invention to accurately predict one or more *in vivo* pharmacokinetic properties of a compound in a mammalian system of interest from input data derived from a selected *in vitro* or *in vivo* data source. The method includes the steps of (i) generating initial adjustment parameter values for one or more independent parameters of the simulation model by utilizing a curve-fitting algorithm to simultaneously fit to the model one or more input variables corresponding to a pharmacokinetic property of a compound test set derived from (a) a first data source corresponding to the mammalian system of interest, and (b) a second data source corresponding to a system other than the mammalian system of interest; (ii) selecting adjustment parameter values that permit correlation of one or more of the input variables from the first data source to one or more input variables from the second data source; (iii) repeating steps (i) and (ii) one or more times for one or more additional independent parameters of the simulation model until deviation of the correlation is minimized; and (iv) utilizing the selected adjustment parameters as constants for the independent parameters in the simulation model.

The present invention further includes a method for producing a pharmacokinetic-based simulation model for use in the PK tool that facilitates estimation of a selected parameter value in a first segment of mammalian system of interest utilizing input data for the selected parameter that corresponds to a second segment of the mammalian system of interest. The method involves (i) providing a logic function module in the simulation model that includes a set of regional correlation parameter values for at least first and second segments of the mammalian system of interest that facilitates estimation of a selected parameter value in the first segment of the mammalian system of interest utilizing input data for the selected parameter that corresponds to the second segment of the mammalian system of interest; and (ii) providing a control statement in the simulation model which initiates the regional correlation estimation function of the logic function module when a value for the first segment is not supplied as input into the model.

The present invention also provides a method for generating formulation profiles for a compound of interest utilizing the PK tool of the invention.

The PK tool of the invention may be provided as a computer system, as an article of manufacture in the form of a computer-readable medium, or a computer program product and the like. Subsystems and individual components of the PK tool also can be utilized and adapted in a variety of disparate applications for predicting the fate of an administered compound. The PK tool and methods of the invention can be used to screen and design compound libraries, select and design drugs, as well as predict drug efficacy in mammals from *in vitro* and/or *in vivo* data of one or more compounds of interest. The PK tool and methods of the invention also finds use in selecting, designing, and preparing drug compounds, and multi-compound drugs and drug formulations (i.e., drug delivery system) for preparation of medicaments for use in treating mammalian disorders.

DEFINITIONS

Absorption: Transfer of a compound across a physiological barrier as a function of time and initial concentration. Amount or concentration of the compound on the external and/or internal side of the barrier is a function of transfer rate and extent, and may range from zero to unity.

Bioavailability: Fraction of an administered dose of a compound that reaches the sampling site and/or site of action. May range from zero to unity. Can be assessed as a function of time.

Compound: Chemical entity.

Computer Readable Medium: Medium for storing, retrieving and/or manipulating information using a computer. Includes optical, digital, magnetic mediums and the like; examples include portable computer diskette, CD-ROMs, hard drive on computer etc. Includes remote access mediums; examples include internet or intranet systems. Permits temporary or permanent data storage, access and manipulation.

Data: Experimentally collected and/or predicted variables. May include dependent and independent variables.

Dissolution: Process by which a compound becomes dissolved in a solvent.

Input/Output System: Provides a user interface between the user and a computer system.

Permeability: Ability of a physiological barrier to permit passage of a substance. Refers to the concentration-dependent or concentration-independent rate of transport (flux), and collectively reflects the effects of characteristics such as molecular size, charge, partition coefficient and stability of a compound on transport. Permeability is substance and barrier specific.

Physiologic Pharmacokinetic Model: Mathematical model describing movement and disposition of a compound in the body or an anatomical part of the body based on pharmacokinetics and physiology.

Production Rule: Combines known facts to produce ("infer") new facts. Includes production rules of the "IF ... THEN" type.

Simulation Engine: Computer-implemented instrument that simulates behavior of a system using an approximate mathematical model of the system. Combines mathematical model with user input variables to simulate or predict how the system behaves. May include system control components such as control statements (e.g., logic components and discrete objects).

Solubility: Property of being soluble; relative capability of being dissolved.

Transport Mechanism: The mechanism by which a compound passes a physiological barrier of tissue or cells. Includes four basic categories of transport: passive paracellular, passive transcellular, carrier-mediated influx, and carrier-mediated efflux.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1 shows schematic of method to generate input data for selected route of administration, mammalian system, and at least one primary barrier to absorption.

Figure 2 shows schematic of method for selecting sampling site relative to administration site and barrier to absorption.

Figure 3 is a high level INPUT/PROCESS/OUTPUT diagram of the PK tool of the invention.

Figure 4 is a high level flow chart and structure chart of the PK tool and method of the invention.

Figure 5 is a graphical diagram illustrating generic compartment-flow simulation model and exemplary symbolic relationships among compartments, flow regulators, converters and input links.

Figure 6 is a key for **Figure 5**.

Figure 7 is a graphical diagram illustrating generic pharmacokinetic first-order two-compartment open plasma model for intravenous injection. D is total drug, V is apparent volume of distribution, and C is drug concentration for either plasma (p) or tissue (t). k12 and k21 represent first-order rate transfer constants for movement of drug from compartment 1 to compartment 2 (k12) and from compartment 2 to compartment 1 (k21). k10 represents first-order rate transfer constant for movement (elimination) of drug from compartment 1 to compartment 0.

Figure 8 is a graphical compartment-flow diagram illustrating the plasma simulation model of Figure 7 and exemplary relationships among compartments, flow regulators, converters and input links.

Figure 9 shows schematic of a method of the invention for development of an initial physiologic-based simulation model for PK tool and method of the invention.

Figure 10 shows schematic of a method of the invention for development of a physiologic-based simulation model having selectively optimized adjustment parameters.

Figure 11 shows graphical compartment-flow diagram illustrating the mass-volume GI tract simulation model of the invention linked to a training/validation plasma model.

Figure 12 illustrates compartment, flow regulator and converter components of the mass-volume GI tract simulation model of the invention.

Figure 13 illustrates structural relationship among compartment and flow regulator components for the mass-volume GI tract simulation model of the invention.

Figure 14 illustrates structural relationship among flow regulator and converter components for the mass-volume GI tract simulation model of the invention.

Figure 15 illustrates converter components for the mass-volume GI tract simulation model of the invention.

Figure 16 compares plasma concentration profiles derived from clinical studies of gancyclovir and simulation using volume GI tract simulation model of the invention.

Figure 17 compares plasma concentration profiles derived from clinical studies of gancyclovir and simulation using mass-volume GI tract simulation model of the invention.

Figure 18 shows graphical compartment-flow diagram illustrating the *in vivo* data analysis-processing IV/PO PK model (intravenous/oral administration) of the invention.

Figure 19 shows schematic of method for development of initial integrated physiologic-based GI tract simulation model of PK tool and method of the invention.

Figure 20 shows graphical compartment-flow diagram illustrating the GI tract fluid transit model component of the PK tool and method of the invention.

Figure 21 shows graphical compartment-flow diagram illustrating the GI tract solubility-dissolution model component of the PK tool and method of the invention.

Figure 22 shows graphical compartment-flow diagram illustrating the GI tract absorption model component of the PK tool and method of the invention.

Figure 23 shows graphical compartment-flow diagram illustrating integration of the GI tract fluid transit model, solubility-dissolution model, and absorption model components for one GI segment of the PK tool and method of the invention.

Figure 24 shows graphical compartment-flow diagram illustrating integrated GI tract simulation model components (without converters or input link connectors) of the PK tool and method of the invention.

Figure 25 shows graphical compartment-flow diagram illustrating integrated GI tract simulation model components (with converters and input link connectors) of the PK tool and method of the invention.

Figure 26 shows schematic of method for development of selectively optimized adjustment parameters and for optimization of the integrated physiologic-based GI tract simulation model of PK tool and method of the invention.

Figure 27 shows schematic of method for selection of model parameters for utilization in a given physiologic-based GI tract simulation model of PK tool and method of the invention.

Figure 28 shows schematic of method for regional (segmental) calculation/estimation of permeability from one or more user input values for permeability of a given GI tract region/segment. Regional permeability (Pe) correlation based on input of Pe value for duodenum is illustrated.

Figure 29 shows graphical converter diagram illustrating volume, surface area, dose, time and pH parameters and calculations for integrated GI tract simulation model components of the PK tool and method of the invention.

Figure 30 shows graphical converter diagram illustrating GI tract transit time parameters and calculations for integrated GI tract simulation model components of the PK tool and method of the invention.

Figure 31 shows graphical converter diagram illustrating GI tract permeability parameters and calculations for integrated GI tract simulation model components of the PK tool and method of the invention.

Figure 32 shows graphical converter diagram illustrating GI tract solubility parameters and calculations for integrated GI tract simulation model components of the PK tool and method of the invention.

Figure 33 shows graphical converter diagram illustrating GI tract control release formulation parameters and calculations for integrated GI tract simulation model components of the PK tool and method of the invention.

Figure 34 shows graphical compartment-converter diagram illustrating GI tract concentration parameters and calculations for integrated GI tract simulation model components of the PK tool and method of the invention.

Figure 35 shows graphical compartment-converter diagram illustrating GI tract dissolution parameters and calculations for integrated GI tract simulation model components of the PK tool and method of the invention.

Figure 36 shows graphical compartment-converter diagram illustrating GI tract output calculations for absorption for integrated GI tract simulation model components of the PK tool and method of the invention.

Figure 37 shows graphical converter diagram illustrating GI tract output calculations for soluble mass absorption rate (flux) for integrated GI tract simulation model components of the PK tool and method of the invention.

Figure 38 shows graphical compartment-flow-converter diagram illustrating GI tract output calculations for cumulative dissolution rate and amount for integrated GI tract simulation model components of the PK tool and method of the invention.

Figure 39 shows graphical compartment-flow-converter diagram illustrating GI tract output calculations for cumulative control release formulation rate and amount for integrated GI tract simulation model components of the PK tool and method of the invention.

Figure 40 illustrates database and rulebase compartment, flow regulator and converter components for the integrated physiologic-based GI tract simulation model of the invention.

Figure 41 illustrates structural relationship among compartment and flow regulator components for the integrated physiologic-based GI tract simulation model of the invention.

Figure 42 illustrates structural relationship among flow regulator and converter components for the integrated physiologic-based GI tract simulation model of the invention.

Figure 43 illustrates structural relationship among converter components for the integrated physiologic-based GI tract simulation model of the invention.

Figure 44 is a high level INPUT/PROCESS/OUTPUT diagram of the PK tool of the invention as presented to a user of the carrying out a method of the invention, with inputs provided by the user and outputs provided by the PK tool.

Figure 45 illustrates a flow chart and structure chart of a subsystem of the PK tool and method of the invention for selection of a physiological GI tract model from a model database and a parameter database.

Figure 46 is a flow chart and structure chart of the system of the PK tool and method of the invention.

Figure 47 is a flow chart and structure chart of a menu of the system of the PK tool and method of the invention.

Figure 48 illustrates correlation of extent of absorption for fraction of the dose absorbed in portal vein (FDp), as predicted using physiologic-based GI tract simulation model and PK tool of the invention, to FDp derived from human clinical data for 12 compounds.

Figure 49 illustrates correlation of rate of absorption for fraction of the dose absorbed in portal vein (FDp), as predicted using integrated physiologic-based GI tract simulation model and PK tool of the invention, to FDp derived from human clinical data for 12 compounds.

Figure 50 compares plasma levels as predicted using integrated physiologic-based GI tract simulation model and PK tool of the invention, to plasma levels derived from human clinical data for a test compound.

Figure 51 compares plasma levels as predicted using integrated physiologic-based GI tract simulation model and PK tool of the invention, to plasma levels derived from human clinical data for a test compound.

Figure 52 compares plasma levels as predicted using integrated physiologic-based GI tract simulation model and PK tool of the invention, to plasma levels derived from human clinical data for a test compound.

Figure 53 shows high level INPUT/PROCESS/OUTPUT diagram of the PK tool of the invention for SAR/QSAR and CAD/CAE compound design and synthesis.

Figure 54 shows high level flow and structure chart for screening method of the invention utilizing the PK tool and method of the invention.

DESCRIPTION OF SPECIFIC EMBODIMENTS

A pharmacokinetic tool (PK tool) and method is provided for predicting absorption of a compound relative to a physiological barrier of a mammalian system of interest, including extravascularly administered compounds. This includes, but is not limited to, prediction of rate, extent and/or concentration of a compound. The mammal is a human or a non-human animal. The method utilizes the PK tool, and optionally separately operable subsystems or components thereof. The PK tool and method of the invention also facilitates prediction of the fate of a compound in a mammal based on absorption and one or more additional bioavailability parameters including distribution, metabolism, elimination, and optionally toxicity.

The PK tool includes as computer-readable components, an input/output system, a physiologic-based simulation model of a mammalian system of interest, and a simulation engine. The input/output system may be any suitable interface between user and computer system, for input and output of data and other information, and for operable interaction with a simulation engine and a simulation model.

Input data into the PK tool and method of the invention is dose, permeability and solubility data for a test compound of interest, and optionally one or more of dissolution rate, transport mechanism, transit time, pH, delivery system rate such as controlled release rate or formulation release rate (delivery system referred to herein as "formulation"), dosing schedule, and simulation run time. The input data may be derived from *in vitro* or *in vivo* sources. *In vitro* data includes tissue and cell and natural and artificial preparations thereof, physicochemical, molecular structure and molecular structure-activity relationship (SAR) and quantitative-SAR (QSAR) data. *In vivo* data includes mammal data. The input data corresponds to one or more given physiological segments/regions of the mammalian system of interest.

The simulation output includes an absorption profile characterized by one or more of rate of absorption, extent of absorption, and concentration of the compound relative to a selected sampling site of interest located across a physiological barrier of the mammalian system of interest, i.e., rate and/or extent of transfer of a test sample from an external site (e.g., apical) across a physiological barrier (e.g., epithelium) to an internal site (e.g., basolateral) of that barrier. This can include prediction of rate, extent and/or concentration of a compound at the site of action when the selected sampling site is the site of action. Transfer rate and/or extent are generated utilizing initial dose data for the test compound and *in vitro* and/or *in vivo* derived data including permeability and solubility data, and optionally dissolution rate and transport mechanism data (i.e., passive paracellular, passive transcellular, carrier-mediated influx, carrier-mediated efflux) for the test compound. Solubility and dissolution rate are interrelated and effect the ability of the compound to be solubilized at a rate sufficient for absorption to occur across a particular membrane. Permeability refers to the concentration-dependent or concentration-independent rate of transport (flux), and collectively reflects the effect of molecular size, charge, partition coefficient and stability of a compound on absorption for a particular physiological barrier, where the physiological barrier(s) depends on the selected route of administration. Molecular size, charge and partition coefficient determines in large part whether a compound is transported via a paracellular or transcellular mechanism. Stability is a general feature that relates to whether the compound remains intact long enough to be absorbed. Together, dose, solubility and permeability data, and optionally dissolution rate and transport mechanism data, are primary bioavailability factors utilized by the PK tool and method of the invention to generate an absorption profile for a test compound of interest.

An absorption profile generated by the PK tool and method of the invention can be uni- or multi-dimensional output that reflects one or more simulated parameters of the mammalian system of interest relative to the sampling site. The sampling site, for example, portal vein, plasma, tissue, organ and the like, is chosen depending on the intended end use of the PK tool and method of the invention. Output of the method and PK tool can be utilized to profile or rank the compound by a selected absorption parameter, and optionally, absorption and one or more additional bioavailability parameters and toxicity.

The simulation engine comprises a differential equation solver and, optionally, a system control statement module. This includes various computer-readable algorithms for numerical iteration of mathematical equations over interval dt and for processing rules, scenarios and the like that direct a simulation.

The simulation model corresponds to a physiologic-based multi-compartment model of a mammalian system of interest, where the mammalian system represents a physiological barrier to absorption that is based on a selected route of administration, i.e., the location at which the compound is introduced to a mammal. More particularly, the physiologic-based simulation model of the PK tool and method of the invention is a mathematical model comprising as operably linked components: (i) differential equations for calculating one or more of fluid transit, fluid absorption, mass transit, mass dissolution, mass solubility, and mass absorption of a test compound for one or more physiological segments of the mammal system of interest; and (ii) initial parameter values for the differential equations corresponding to physiological parameters and selectively optimized adjustment parameters, and optionally one or more regional correlation parameters, for one or more physiological segments of the mammal system of interest; and optionally (iii) control statement rules for one or more of absorption, permeability, solubility, dissolution, concentration, and mathematical error correction, for one or more physiological segments of the mammal system of interest. The simulation model also may include one or more smoothing functions that facilitate calculation of transitional parameter values occurring between one or more of the physiological segments.

The differential equations of a selected simulation model of a mammalian system of interest describe the rate processes of absorption, and optionally other events, of that model, which in turn describe compound concentrations in the system as a function of time. (See, e.g., Shargel et al., *Applied Biopharmaceutics and Pharmacokinetics*, Appelton & Lange, East Norwalk, Conneticut, 1993). Thus, the differential equations are selected for a particular model.

The initial physiological parameter values of a given simulation model can be generated de novo or obtained from existing sources including the literature. The selectively optimized adjustment parameter values of a given simulation model of the invention represent regression or stochastic analysis derived values that are used as constants for one or more independent parameters of the model. In particular, the selectively optimized adjustment parameter values are obtainable by using a stepwise fitting and selection process that employs regression- or stochastic-based curve-fitting algorithms to simultaneously estimate the change required in a value assigned to an initial absorption parameter of the model in order to change an output variable. The input variables utilized for fitting include a combination of *in vitro* data (e.g., permeability, solubility) and *in vivo* pharmacokinetic data (e.g., fraction of dose absorbed, plasma levels) for a compound test set having compounds exhibiting a diverse range of *in vivo* absorption properties. Thus, the input variables used for regression- or stochastic-based fitting are derived from (a) a first data source corresponding to the mammalian system of interest (e.g., *in vivo* pharmacokinetic data from human for the compound test set), and (b) a second data source corresponding to a system other than the mammalian system of interest (e.g., *in vitro* solubility data and *in vitro* permeability data from rabbit tissue for the compound test set). A fitted adjustment parameter value for a given independent parameter is then selected that, when supplied as a constant in the model, permits correlation of one or more of the input variables from the first data source to one or more input variables from the second data source. The process is repeated one or more times for one or more additional independent parameters of the simulation model until deviation of the correlation is minimized. These "selectively optimized" adjustment parameters are then provided to a given simulation model as constants or ranges of constants or functions that modify the underlying equations of the model. The selectively optimized adjustment parameters facilitate accurate correlation of *in vitro* data derived from a particular type of assay corresponding to the second data source (e.g., Caco-2 cells, segment-specific rabbit intestinal tissue sections etc.) to *in vivo* absorption for a mammalian system of interest corresponding to the first data source (e.g., segment-specific portions of the human GI tract) for diverse test sample data sets. Selectively optimized adjustment parameters also can be utilized to facilitate accurate correlation of *in vivo* data derived from a first species of mammal (e.g., rabbit) to a second species of mammal (e.g., human).

For a simulation model representing two or more anatomical segments of a given mammalian system, the model will preferably include regional correlation parameters. The regional correlation parameters permit estimation of a selected parameter value for a first segment of the mammalian system from correlation using a value of the selected parameter for a second segment of the mammalian system. The regional correlation parameters represent a collection of empirically derived values or selectively optimized adjustment parameter values for various segments of the mammalian system of interest, for example, permeability values. The regional (i.e., segmental) correlation is performed by logic function of the model, which when activated utilizes a function/transformation algorithm to estimate the parameter value for the second segment from (1) the corresponding regional correlation parameters, and (2) a user provided input value for the same parameter, but for a different segment. The regional correlation logic function of the model is activated when a user does not supply an input value for a particular parameter. For example, when a user of the PK tool supplies a single permeability value as input into a GI tract simulation model of the invention, such as a permeability value derived from Caco-2 cells that corresponds to colon, then regional permeability correlation is performed by the PK tool to estimate permeability in the other GI tract segments, such as duodenum, jejunum, and ileum.

The control statement rules include various logic elements utilized for providing guidance as to how a given simulation is to proceed. For instance, a control statement rule would include "IF ... THEN" production rules. An example of a production rule would be "IF solubility of compound is zero THEN absorption is zero." The production rules are based on rules of thumb (heuristics) and the like, and may be generated by correlation of parameters and simulation runs. Rules can be added, modified or removed to change how a simulation model responds to incoming data.

The input/output system, simulation engine and simulation model of the PK tool are capable of working together to carry out the steps of (1) receiving as input data, the initial dose of a test compound at the site of administration and permeability and solubility, and optionally dissolution rate and transfer mechanism data; and (2) applying the simulation engine and the simulation model to generate as output data a simulated *in vivo* absorption profile for the test compound that reflects rate, extent and/or concentration of the test sample at a given sampling site for a selected route of administration in a mammalian system of interest. This includes uni- and multi-dimensional output profiles that collectively reflect parameters of absorption, which can be directly or indirectly utilized for characterizing *in vivo* absorption, as well as one or more additional bioavailability parameters including distribution, metabolism, elimination, and optionally toxicity.

The selected routes of administration include enteral (e.g., buccal or sublingual, oral (PO), rectal (PR)), parenteral (e.g., intravascular, intravenous bolus, intravenous infusion, intramuscular, subcutaneous injection), inhalation and transdermal (percutaneous). The preferred route of administration according to the method of the invention is oral administration. The selected route of administration determines the type and/or source of assay or structure-property parameters employed for obtaining a set of input data utilized for generating a simulated *in vivo* absorption profile. That is, artificial, cell or tissue preparations and the like derived from or representative of a physiological barrier to absorption for a selected route of administration are chosen to generate the relevant input data for use as input into the PK tool. For instance, input data for simulating fate of a test sample following oral administration can be based on cell culture and/or tissue assays that employ biological preparations derived from or representative of the gastrointestinal tract of a mammal of interest, e.g., gastrointestinal epithelial cell preparations for permeability and transfer mechanism data, and physiological/anatomical fluid and admixing conditions corresponding to the relevant portions of the gastrointestinal tract for solubility and dissolution rate assays. Assays for collecting input data for specialized physiological barriers such as the blood brain barrier may initially assume intravascular delivery and thus instantaneous absorption as a first step. In this situation an assay is selected to generate input data relative to the blood brain barrier, which include for instance cell culture and/or tissue assays that employ biological preparations derived from or representative of the interface between systemic blood and the endothelial cells of the microvessels of the brain for a mammal of interest, e.g., blood-brain-barrier microvessel endothelial cell preparations for permeability and transfer mechanism data, and physiological/anatomical fluid and admixing conditions corresponding to the relevant portions of the blood membrane barrier for solubility and dissolution rate assays. A series of assays may be employed to collect input data for two or more barriers to absorption. As an example, oral, hepatic, systemic and blood brain barrier assays may be utilized to obtain input data for screening compound libraries for orally delivered compounds that target brain tissue.

The sampling site relates to the point at which absorption parameters are evaluated for a test sample of interest. This is the site at which rate, extent and/or concentration of a test sample is determined relative to a selected site of administration, and is separated from the site of administration by at least one physiological barrier to absorption. For generating simulated absorption profiles, the sampling site preferably is separated from the site of administration by an individual primary barrier to absorption, which can be utilized to evaluate additional absorption events by secondary barriers to absorption so as to sequentially and collectively reflect the summation of absorption events at other sampling sites of interest. As an example, the sampling site selected for oral delivery may be the portal vein where the primary barrier to absorption is the gastrointestinal lumenal membrane, or systemic blood where a secondary barrier to systemic absorption is the liver after the test sample passes from the portal vein through the liver to systemic circulation. Thus the type of physiological barrier(s) residing between a site of administration and a sampling site reflects the type of assay(s) employed for generating the desired input data for use as input data into the PK tool of the invention.

As the selected route of administration determines the barrier(s) to absorption and the physiological parameters that affect absorption events following administration, it also determines the physiologic-based pharmacokinetic simulation model employed in the PK tool for generation of the simulated *in vivo* absorption profile. By way of example, if the proposed route of administration is oral, then a primary barrier to absorption is the lumenal membrane of the gastrointestinal tract, and a secondary event affecting systemic bioavailability is first pass metabolism by the liver. Thus, a given simulation model and its associated parameters for simulating the fate of a compound selected for oral delivery is chosen to represent this scenario. The model would include therefore relevant components of the gastrointestinal tract for administration and absorption (i.e., stomach, duodenum, jejunum, ileum, and colon) and a primary sampling site (i.e., portal vein) from which to evaluate a primary absorption event. In this instance a secondary barrier to absorption for oral delivery is the liver and a secondary sampling site is systemic blood/plasma. This basic approach to choosing a physiologic-based pharmacokinetic model also applies to models employed to simulate absorption by target organs and the like, where a physiological barrier to absorption is the tissue and/or membrane separating systemic blood from the target organ, such as the blood brain barrier. In this situation if oral delivery is selected as the preferred route of administration for a compound targeting brain tissue, then a gastrointestinal tract model and blood brain barrier model may be implemented separately and/or combined through a complementary plasma component of the models for screening purposes.

The physiological models are selected from a repository of delivery route-specific models stored in a memory, a database, or created de novo. Physiological models of the invention include those corresponding to common routes of administration or barriers to absorption, such as oral (GI tract), ocular (eye), transdermal (skin), rectal, intravenous, rectal, subcutaneous, respiratory (nasal, lung), blood brain barrier and the like. For constructing a model de novo, the basic approach is to identify and isolate a primary barrier to a specific absorption event from secondary events so that each barrier to absorption can be tested and validated in isolation. This involves selecting a site of administration that is separated from a sampling site by a primary physiological barrier to absorption and then building a developmental physiological model that incorporates rate process relations and limitations to describe the isolated absorption event. If desired, the secondary events can be added sequentially so that each additional layer of complexity to the model can be tested and validated in isolation from other components of the initial model.

The invention also relates to a method and PK tool for designing compounds based on absorption. This aspect of the invention utilizes output of the method and PK tool as the input to a structure-activity relationship (SAR) or quantitative SAR (QSAR) design/selection process, e.g., a SAR and/or QSAR computer-assisted design/engineering/selection (CAD/CAE (collectively "CAD")) process. Output of the CAD process is then optionally used as input for the method and PK tool of the invention. SAR and QSAR information may then be incorporated into a database for subsequent iterative design and selection in the CAD process. For instance, compounds designed using a CAD process may be tested *in vitro* and/or *in vivo* for absorption parameters such as permeability, solubility, dissolution, and transport mechanism, and optionally one or more additional bioavailability parameters, and the data employed as input into the PK tool and method of the invention (i.e., iterative design). Alternatively, the parameters can be predicted from SAR or QSAR information and utilized as input for the method and PK tool of the invention. In this aspect of the invention, the user also is allowed to vary input parameters for "What if" analysis.

In the forward mode of operation, the user can predict absorption, individual parameters of absorption, as well as one or more other bioavailability parameters of a compound from relatively few input variables including dose, permeability and solubility. Additionally, the user can evaluate alternatives by changing any of the parameters and constants of the system, and observe the ripple effect of the change in one or more parameters on all other parameters. For instance, the user can evaluate alternative absorption profiles using "What if" analysis with any parameter of the system.

In the backward mode of operation, the user specifies one or more objective absorption parameters of a formulation of interest and the PK tool and method of the invention generates alternatives to satisfy the objective. In this aspect of the invention, well-defined properties of the compound (and the formulation base minus the compound) are utilized by the PK tool and method to evaluate alternative dosing and formulation profiles for a given compound. The user also is allowed to vary input dosing and formulation parameters for "What if" analysis. Simulated absorption profiles can then be utilized for preparing suitable formulations and/or dosing regimes. Solubility, permeability, doses and the like also may be estimated in the backward mode of operation.

The PK tool and method of the invention is exemplified by physiologic-based simulation model for predicting oral absorption of a compound in one or more segments of the GI tract of a mammal. The segments include the stomach, duodenum, jejunum, ileum, and colon. The simulation model includes differential equations for calculating one or more of fluid transit, fluid absorption, mass transit, mass dissolution, mass solubility, and mass absorption for one or more segments of the GI tract of a mammal of interest. It also includes initial parameter values for the differential equations that correspond to physiological parameters and selectively optimized adjustment parameters for one or more segments of the GI tract of the mammal of interest. The initial parameter values of simulation model also include one or more regional correlation parameter values, which are optional, but preferred for inclusion. The simulation model of the GI tract also includes control statement rules for one or more of transit, absorption, permeability, solubility, dissolution, concentration, and mathematical error correction for one or more segments of the GI tract of the mammal of interest.

The physiologic-based simulation model of the GI tract corresponds to a compartment-flow simulation model of the GI tract of a mammal characterized by one or more of fluid volume, fluid absorption, insoluble mass, soluble mass, and soluble mass absorption compartments. The compartments of the compartment-flow simulation model are operably linked by one or more flow regulators characterized by fluid absorption rate, fluid volume transit rate, insoluble mass transit rate, insoluble mass dissolution rate, soluble mass transit rate, and soluble mass absorption rate. The flow regulators of the compartment-flow simulation model are modified by one or more converters characterized by fluid volume, fluid volume absorption rate constant, fluid volume transit rate constant, insoluble mass, insoluble mass transit rate constant, dissolution rate constant, soluble mass, soluble mass transit rate constant, surface area, dissolved mass concentration and permeability.

The PK tool and method of this invention accelerate selection and design of compounds for treatment of mammalian disorders, allowing same day response time. The invention optimizes the drug development process in terms of bioavailability parameters, and uses simple *in vitro* parameters for predicting the *in vivo* fate of an administered compound. The PK tool and method of the invention also permits utilization of *in vivo* data from one type of mammal (e.g. rabbit) to predict absorption in a different type of mammal (e.g. human). The invention also is particularly well suited for iterative selection and design of compounds based on structure-bioavailability relationships using a SAR/QSAR approach. This reduces total drug development time, and optimizes the drug design and selection process for animal studies and human clinical trials. Moreover, the PK tool and method of the invention allows separate or concurrent consideration of bioavailability parameters and/or biological drug-receptor activity early in the drug development process. The invention also permits a broad range of *in vitro* to interspecies correlation, thereby facilitating optimal selection of an animal model for drug development.

PK Tool and System:

The PK tool of the invention is utilized to generate a simulated *in vivo* absorption profile from dose, solubility and permeability data, and optionally *in vitro* dissolution rate and transport mechanism data for a test compound. The PK tool includes as computer-readable components, an input/output system suitable for data input and data output, a simulation engine having a differential equation solver, and a physiologic-based simulation model comprising a pharmacokinetic model of the mammalian system to be simulated. *In vitro* or *in vivo* data for the test compound is provided through the input/output system, and then the simulation engine and simulation model are applied to facilitate a simulation run so as to generate a user selected *in vivo* absorption profile for the test sample. Together, the simulation engine and simulation model are employed to simulate the fate of a test sample in the system under investigation.

The PK tool is based on a compartment-flow simulation model system. The compartment-flow model employs compartments, flow regulators, and converters that collectively regulate flow among the compartments. The model components are represented by a series of differential equations which when run through the simulation engine are solved at each time increment dt based on the initial underlying values of the equations, the input values supplied by the user, and calculations performed by various subsystems of the model when activated in a particular scenario.

The PK tool optionally comprises a repository of different pharmacokinetic models and initial parameter values for a given model. The repository preferably resides in a database of the PK tool, and/or is accessible through an acquisition module. The PK tool also may include one or more curve-fitting algorithms for generation of absorption parameters and constants for correlation of *in vitro* data to *in vivo* data, or *in vivo* data from one species of a mammal to *in vivo* data of a second species of mammal based on a selected route of administration. The curve-fitting algorithms include regression-based and stochastic-based algorithms.

1. Input/Output System

With regard to the components of the PK tool, the input/output system provides a user interface between the user and the PK tool of the invention. The input/output system may be any suitable interface between user and computer system, for input and output of data and other information, and for operable interaction with a simulation engine and a simulation model. For instance, the input/output system may provide direct input form measuring equipment. The input/output system preferably provides an interface for a standalone computer or integrated multi-component computer system having a data processor, a memory, and a display. Input into the method and PK tool of the invention is *in vitro* or *in vivo* data derived from an assay corresponding to a selected route of administration and mammalian system of interest. For example, the user enters the initial parameter values for a test compound, e.g., dose, permeability, and solubility derived from the assay, and then optionally indicates the transport mechanism, e.g., passive transcellular, passive paracellular, carrier-mediated influx, or carrier-mediated efflux. When transport mechanism is not indicated, the PK tool can be designed to employ a default transport mechanism, such as passive transcellular. When set to the paracellular mechanism, the absorption of the compound is adjusted to compensate for the lower surface area available for absorption via the paracellular pathway. The model also may incorporate an operation by which the mechanism of absorption can be predicted using the permeability, solubility, molecular structure or other information. This allows the model to automatically compensate for paracellular and/or other absorption mechanisms without requiring prior input and knowledge from the user. Depending on the objective, the user also may specify the pH, delivery system rate such as controlled release rate or formulation release rate (delivery system referred to herein as "formulation"), dosing schedule, and simulation run time, as well as physiologic system specific parameters such as GI transit time when a GI tract model is employed. If values for these parameters are not entered, the PK tool provides default values.

Data may be entered numerically, as a mathematical expression or as a graph that represents a physiological or pharmacokinetic parameter, or alpha such as transcellular, paracellular, passive, active, etc. An advantage of entering data as a graph is that it removes any requirement to define the mathematical relationship between a dependent and an independent variable. The interface output displays and/or compares parameters related to absorption, such as graphs or tables corresponding to rate of absorption, extent of absorption, and concentration profiles, and the like.

The absorption parameters include concentration, rate and/or extent of absorption of a test sample. As can be appreciated, absorption parameters can be represented in multiple different ways that relate time, mass, volume, concentration variables, fraction of the dose absorbed and the like. Examples include rate "dD/dt" and "dc/dt" (e.g., mass/time-mg/hr; concentration/time-μg/ml/hr), concentration "C" (e.g., mass/volume-μg/ml), area under the curve "AUC" (e.g., concentration • time, μg • hr/ml), and extent/fraction of the dose absorbed "F" (e.g., no units, 0 to 1). Other examples include the maximum concentration (C_{max}), which is the maximum concentration reached during the residence of a compound at a selected sampling site; time to maximum concentration (T_{max}), which is the time after administration when the maximum concentration is reached; and half-life ($t_{1/2}$), *e.g.*, the time where the concentration reaches ½ its maximum at a selected sampling site. Other examples of output include individual simulated parameters such as permeability, solubility, dissolution, and the like for individual segments, as well as cumulative values for these and/or other parameters.

2. Simulation Engine

The simulation engine comprises a differential equation solver that uses a numerical scheme to evaluate the differential equations of a given physiologic-based simulation model of the invention. The simulation engine also may include a system control statement module when control statement rules such as IF...THEN type production rules are employed. The differential equation solver uses standard numerical methods to solve the system of equations that comprise a given simulation model. These include algorithms such as Euler's and Runge-Kutta methods. Such simulation algorithms and simulation approaches are well known (See, e.g., Acton, F.S., *Numerical Methods that Work*, New York, Harper & Row (1970); Burden et al., *Numerical Analysis*, Boston, MA, Prindle, Weber & Schmidt (1981); Gerald et al., Applied Numerical Analysis, Reading, MA, Addison-Wesley Publishing Co., (1984); McCormick et al., Numerical Methods in Fortran, Englewood Cliffs, NJ, Prentice Hall, (1964); and Benku, T., The Runge-Kutta Methods, BYTE Magazine, April 1986, pp. 191-210).

Many different numerical schemes exist for the evaluation of the differential equations. There are literally 100's of schemes that currently exist, including those incorporated into public commercially available computer applications, private industrial computer applications, private individually owned and written computer applications, manual hand-calculated procedures, and published procedures. With the use of computers as tools to evaluate the differential equations, new schemes are developed annually. The majority of the numerical schemes are incorporated into computer applications to allow quick evaluation of the differential equations.

Computer application or programs described as simulation engines or differential equation solver programs can be either interpretive or compiled. A compiled program is one that has been converted and written in computer language (such as C++, or the like) and are comprehendible only to computers. The components of an interpretive program are written in characters and a language that can be read and understood by people. Both types of programs require a numerical scheme to evaluate the differential equations of the model. Speed and run time are the main advantages of using a compiled rather than a interpretive program.

A preferred simulation engine permits concurrent model building and simulation. An example is the STELLA® program (High Performance Systems, Inc.). STELLA® is an interpretive program that can use three different numerical schemes to evaluate the differential equations: Euler's method, Runge-Kutta 2, or Runge-Kutta 4. The program KINETICA™ (InnaPhase, Inc.) is another differential equation solving program that can evaluate the equations of the model. By translating the model from a STELLA® readable format to a KINETICA™ readable format, physiological simulations can be constructed using KINETICA™, which has various fitting algorithms. This procedure can be utilized when the adjustment parameters are being optimized in a stepwise fashion.

3. Simulation Model

The simulation model is a mathematical model of a multi-compartment physiological model of a mammalian system (e.g., GI tract) that corresponds to the selected route of administration (e.g., oral). A given physiological model is represented by series of differential equations that describe rate process interactions among anatomical segments for the physiological system under investigation. The individual segments or compartments are represented mathematically as a one, two and/or three compartment kinetic system. The segments are linked in a stepwise fashion so as to form an integrated physiological model describing absorption of a compound relative to the anatomical segments and at least one sampling site for assessing an absorption event in isolation. For a model simulating oral delivery, anatomical segments of the GI tract are provided, which can include the stomach, duodenum, jejunum, ileum and colon. A sampling site for the GI tract may be the portal vein and/or plasma. The rectum and colon would be applicable for modeling a rectal route of delivery. Segments and sampling site for buccal or sublingual delivery routes can include the mouth, cheek/tongue tissue and plasma. For ocular routes, this can include aqueous humor, conjunctival sac, tear duct, nasal cavity and plasma. For the lung routes, this can include respiratory bronchioles zone and plasma. For delivery via the nose, this can include nasal cavity and plasma. For the topical and transdermal routes, this can include epidermal, dermal, subcutaneous tissue, muscle and plasma. Other systems adhere to these basic designs.

Of course compartments representing a particular anatomical segment can be added or removed depending on the model's intended end use, such as when an isolated segment is examined, or when it is desired to account for parameters affecting bioavailability at additional sampling sites. For example, compartments can be added to account for both pre- or post-absorptive protein binding or complex formation to account for reversible association of a compound to the proteins (albumin and a1-acid glycoprotein) of blood, or more usually plasma. Other compartments that may be added would include those that account for phase I and/or phase II hepatic metabolism. Formulation compartments that account for variable compound formulations also can be added, such as time-release, extended release or otherwise controlled release formulations. Another example is inclusion of kidney compartments to account for renal clearance.

The compartments can be modified by factors that influence absorption such as mass, volume, surface area, concentration, permeability, solubility, fluid secretion/absorption, fluid transit, mass transit and the like, depending on the physiological system under investigation. As a rule of thumb, compartment modifiers relate to input variables. For instance, where transport mechanism and dissolution rate are variables considered for generating an absorption profile, then the physiological model will include compartments and parameters that account for these variables.

When represented as a compartment-flow simulation model, the anatomical segments of a physiological model typically include one or more central and peripheral compartments that reversibly communicate through a flow regulator. A central compartment represents the interior or mucosal side of an anatomical segment. A peripheral compartment represents the blood side of the segment. The central and peripheral compartments are connected by a flow regulator representing a physiological barrier through which material from the central compartment "flows" or is transferred to the peripheral compartment at some empirically defined or calculated transfer rate "k12" applied by a converter, which allows calculation of parameters using compartment values. Transfers ("flows") between compartments can be zero order, first order, second order and/or mixed order processes. As an example, a first order transfer from central compartment 1 to peripheral compartment 2 can be defined by a finite difference equation connecting inputs (e.g., rate constant k12 and amount of compound in central compartment = amount + dt*(-elimination – k12+ k21)) to the flow controller between the compartments (e.g., k12) and setting it as the product of the two variables. Thus the underlying equations of the model are utilized to calculate the amount of a compound in each compartment, and standard differential equations interrelate the system of compartments and their equations. This permits the model to simulate movement of a compound through each compartment according to the calculated rates at each time increment (dt). Since all movement between compartments is in units of mass, the blood side and transferred test compound concentration is calculated from the amount of compound in the blood side (peripheral compartment) and volume of the mucosal side (central compartment). A model cycle is entered through the input/output user interface as incremental pulses (to simulate ramp, plug flow/lag times) or as a fixed time range to initiate and effectuate cycling of a test compound of interest.

The basic structure of a physiological model and mathematical representation of its interrelated anatomical segments can be constructed using any number of techniques. The preferred techniques employ graphical-oriented compartment-flow model development computer programs such as STELLA®, Kinetica™ and the like. Many such programs are available, and most employ graphical user interfaces for model building and manipulation. In essence, symbols used by the programs for elements of the model are arranged by the user to assemble a diagram of the system or process to be modeled. Each factor in the model may be programmed as a numerical constant, a linear or non-linear relationship between two parameters or as a logic statement. The model development program then generates the differential equations corresponding to the user constructed model. For example, STELLA® employs five basic graphic tools that are linked to create the basic structure of a model: (1) stocks; (2) flows; (3) converters; (4) input links; and (5) infinite stocks (See, e.g., Peterson *et al.*, STELLA® II, Technical Documentation, High Performance Systems, Inc., (1993)). Stock are boxes that represent a reservoir or compartment. Flows or flow regulators control variables capable of altering the state of compartment variables, and can be both uni- and bi-directional in terms of flow regulation. Thus, the flow/flow regulators regulate movement into and out of compartments. Converters modify flow regulators or other converters. Converters function to hold or calculate parameter variable values that can be used as constants or variables which describe equations, inputs and/or outputs. Converters allow calculation of parameters using compartment values. Input links serve as the internal communication or connective "wiring" for the model. The input links direct action between compartments, flow regulators, and converters. In calculus parlance, flows represent time derivatives; stocks are the integrals (or accumulations) of flows over time; and converters contain the micro-logic of flows. The stocks are represented as finite difference equations having the following form: Stock(t) = Stock(t-dt) + (Flow)*dt. Rewriting this equation with timescripts and substituting t for dt: $\text{Stock}_t = \text{Stock}_{t-\Delta t} + \Delta t^*(\text{Flow})$. Re-arranging terms: $(\text{Stock}_t - \text{Stock}_{t-\Delta t})/\Delta t = \text{Flow}$, where "Flow" is the change in the variable "Stock" over the time interval "t." In the limit as Δt goes to zero, the difference equation becomes the differential equation: d(Stock)/dt = Flow. Expressing this in integral notation: $\text{Stock} = \int \text{Flow}\ dt$. For higher-order equations, the higher-order differentials are expressed as a series of first-order equations. Thus, computer programs such as STELLA® can be utilized to generate physiologic-based multi-compartment models as compartment-flow models using graphical tools and supplying the relevant differential equations of pharmacokinetics for the given physiologic system under investigation. An example of iconic tools and description, as well as graphically depicted compartment-flow models generated using STELLA® and their relation to a conventional pharmacokinetic IV model is illustrated in **Figures 5-8**.

The model components may include variable descriptors. Variable descriptors for STELLA®, for example, include a broad assortment of mathematical, statistical, and built in logic functions such as boolean and time functions, as well as user-defined constants or graphical relationships. This includes control statements, e.g., AND, OR, IF … THEN ... ELSE, delay and pulsing, that allow for development of a set of production rules that the program uses to control the model. Variable descriptors are inserted into the "converters" and connected using "input links." This makes it is possible to develop complex rule sets to control flow through the model. The amount of time required to complete one model cycle is accomplished by inputting a total run time and a time increment (dt). The STELLA® program then calculates the value of every parameter in the model at each successive time increment using Runge-Kutta or Euler's simulation techniques. The preferred simulation technique is Runge-Kutta. Once a model is built, it can be modified and further refined, or adapted or reconstructed by other methods, including manually, by compiling, or translated to other computer languages and the like depending on its intended end use.

A preferred method of the invention for constructing a physiological model for *in vivo* prediction from *in vitro* input data is depicted in **Figure 9**. This method employs a two-pronged approach that utilizes a training set of standards and test compounds having a wide range of dosing requirements and a wide range of permeability, solubility, transport mechanisms and dissolution rates to refine the rate process relations and generate the initial values for the underlying equations of the model. The first prong employs the training/validation set of compounds to generate *in vivo* pharmacokinetic data (e.g., human plasma profiles). The second prong utilizes the training/validation set of compounds to generate *in vitro* permeability, solubility, transport mechanism and dissolution rate data that is employed to perform a simulation with the developmental physiological model. The *in vivo* pharmacokinetic data is then compared to the simulated *in vivo* data to determine how well a developmental model can predict the actual *in vivo* values from *in vitro* data. The developmental model is adjusted until it is capable of predicting *in vivo* absorption for the training set from *in vitro* data input. Then the model can then be validated using the same basic approach and to assess model performance.

In particular, three primary sets of data are generated from the training set for the comparison. The first set of data is empirically derived *in vivo* plasma data from animals or humans. The second set of data is obtained from conversion of the *in vivo* plasma data to a form corresponding to the primary sampling site of the developmental physiological model. The third set of data is empirically derived *in vitro* data including permeability, solubility, dissolution rate and transport mechanism data. The raw data points are preferably collected and statistically analyzed to provide the best fit data. The best fit data may be obtained by any number of curve-fitting approaches, including standard regression techniques.

The *in vivo* plasma data is utilized to judge how well a developmental simulation model is able to predict absorption of the training set of compounds relative to the empirically derived *in vivo* plasma values. Plasma data also is utilized to calculate absorption at the relevant primary sampling site of the developmental physiological model. For instance, in order to use *in vivo* plasma data in a developmental physiological model, the plasma data must first be converted to data corresponding to the primary sampling site of the model. If plasma is the primary sampling site then no conversion is needed. However, if plasma is not the primary sampling site, then a pharmacokinetic training/validation model relating the primary sampling site and the *in vivo* plasma data is utilized. For example, when the developmental model is of the gastrointestinal tract, the portal vein can be selected as a primary sampling site and plasma selected as a secondary sampling site. Thus in this instance the *in vivo* plasma data is converted to portal vein data so that the parameters affecting secondary bioavailability events are separated from the primary absorption event resulting from passage of the test sample across the gastrointestinal lumen. This is accomplished by adding a plasma-portal vein conversion/validation model that relates *in vivo* plasma data to portal vein data. This plasma-portal vein conversion/validation model can be separate or integrated with the developmental model. In most cases, the plasma-portal vein model is based on a standard central-peripheral pharmacokinetic compartment approach for data conversion. The third set of data, the *in vitro* derived data, is utilized to run the developmental model, and the simulated absorption profile from this data set is compared to the *in vivo* derived plasma and simulated sampling site data. The developmental physiological model is modified until the simulated absorption profiles are in agreement with the *in vivo* derived plasma and simulated sampling site data.

As the number of parameters for evaluation increase it becomes more important to isolate and test each component of the model building process by validation using a standard validation set of compounds. The validation set of compounds should contain a diverse set of compounds that represent a broad range of absorption profiles for which both *in vitro* permeability, solubility, dissolution rate, and transport mechanism data, and *in vivo* plasma data is available. Statistical criteria such as sum of squares of the deviations between experimental data and calculated values obtained from the developmental physiological model are used to determine how well the model fits the data. If the developmental physiological model does not predict a good fit for the data, then the model is adjusted by isolating or including additional rate processes by an iterative approach.

Parameter values utilized in the underlying equations of a given physiological model may be provided in a database for ready access and manipulation by the PK tool of the invention, or provided with a model. The parameter values may include values for physiological parameters, such as rate constants and various other values employed in the PK tool. The rate constants correspond to time-dependent (or time-independent) numerical constants describing rate processes (e.g., k12 and k21). The physiological parameters include rate constants, permeability, solubility, transport mechanism and dissolution rate variables, and the like, as well as pH, volume, surface area, transit times, transit rates, and the like, that are based on the physiology of a given anatomical segment represented in a selected physiological model.

To account for differences between *in vitro* and *in vivo* conditions, as well as differences between *in vivo* conditions of different type of mammals, adjustment parameters that modify one or more of the underlying equations of given simulation model can be utilized to significantly improve predictability. The adjustment parameters include constants or ranges of constants that are utilized to correlate *in vitro* input parameter values derived from a particular *in vitro* assay system (e.g., rabbit intestinal tissue, Caco-2 cells) to a corresponding *in vivo* parameter value employed in the underlying equations of a selected physiological model (e.g., human GI tract). The adjustment parameters are used to build the correlation between the *in vitro* and *in vivo* situations, and *in vivo* (species 1) to *in vivo* (species 2). These parameters make adjustments to the equations governing the flow of drug and/or calculation of parameters. Generally, the parameters are geometric scaling parameters, as exemplified by the general equations described below for a GI tract simulation model of the invention. This aspect of the invention permits modification of existing physiologic-based pharmacokinetic models as well as development of new ones so as to enable their application for diverse compound data sets.

The adjustment parameters of the PK tool and method of the invention are obtainable from iterative rounds of simulation and simultaneous "adjustment" of one or more empirically derived absorption parameters (e.g., physiological parameters for different anatomical segments) until the *in vitro* data from a given type of assay (e.g., Caco-2 cell data) can be used in the model to accurately predict *in vivo* absorption in the system of interest (e.g., human GI). In particular, the adjustment parameters are obtained by a stepwise selective optimization process that employs a curve-fitting algorithm that estimates the change required in a value assigned to an initial absorption parameter of a developmental physiological model in order to change an output variable corresponding to the simulated rate, extent and/or concentration of a test sample at a selected site of administration for a mammalian system of interest. The curve-fitting algorithm can be regression- or stochastic-based. For example, linear or non-linear regression may be employed for curve fitting, where non-linear regression is preferred. Stepwise optimization of adjustment parameters preferably utilizes a concurrent approach in which a combination of *in vivo* pharmacokinetic data and *in vitro* data for a diverse set of compounds are utilized simultaneously for fitting with the model. A few parameters of the developmental physiological model are adjusted at a time in a stepwise or sequential selection approach until the simulated absorption profiles generated by the physiological model for each of the training/validation compounds provides a good fit to empirically derived *in vivo* data. An example of this approach is depicted in **Figures 10** and **26**. Utilization of adjustment parameters permits predictability of diverse data sets, where predictability ranges from a regression coefficient (r^2) of greater than 0.40, 0.45, 0.50, 0.55, 0.60, 0.65, 0.60, 0.65, 0.70, or 0.75 for 80% of compounds in a compound test set having a diverse range of dose requirements and a diverse range of permeability, solubility and transport mechanisms. The preferred predictability ranges from a regression coefficient (r^2) of greater than 0.60, with a regression coefficient (r^2) of greater than 0.75 being more preferred, and greater than 0.80 being most preferred. Adjustment parameters utilized for *in vivo* to *in vitro* prediction (e.g. dog to human) employs the same basic approach.

The regional correlation parameters of the PK tool include constants or ranges of constants that are utilized to estimate a selected parameter value of a first segment of the mammalian system under investigation when that value is not supplied by the user. The model performs this estimation by utilizing a function/transformation algorithm (e.g., utilizing polynomial, exponential, logarithm, or any other variety of transformation approaches) in which (1) regional correlation parameter values, and

(2) one or more values for the parameter that is supplied by the user for a second segment of the mammalian system, are utilized to estimate the value for the first segment. The regional correlation parameters may be empirically derived values or adjustment parameter values for various segments of the mammalian system of interest such as for permeability. A preferred regional correlation approach employs a polynomial-based correlation. The polynomial is based on the particular parameter to be estimated. The regional correlation is performed by logic function of the model, which when activated utilizes the function/transformation algorithm to perform the estimation. The regional correlation logic function of the model is activated when a value is missing for the selected parameter. The estimated value(s) are then utilized as input variables for the particular parameter in question. The model then proceeds by employing the estimated value for subsequent simulation. Various regional correlation parameters can be used, such as permeability, solubility, dissolution rate, transport mechanism and the like. The preferred correlation parameters are for permeability. This permits the PK tool of the invention to predict absorption of a test sample from minimal input permeability values, such as when the simulation model is a GI tract simulation model and when cell-based assays are employed to provide permeability data corresponding to a given GI segment (e.g., Caco-2 cells and colon).

The above described methodology for *in vivo* prediction from *in vitro* input also is followed for *in vivo* prediction for a first species of mammal from *in vivo* input data derived from a second species of mammal.

Since the parameter values are specific for a given physiological model (e.g., GI model-parameters, Ocular model-parameters, Blood-Brain-Barrier-parameters, etc.), parameter values are chosen accordingly. These values are obtainable de novo from experiments or from the literature, and adjustment parameters and regional correlation parameters derivable therefrom. The preferred values are based on a diverse collection of training/validation compounds for which *in vivo* pharmacokinetic data is available.

The various physiological models also may reside in a database, in part or in whole, and may be provided in the database with or without the initial parameter values. The database will preferably provide the differential equations of the model in a compartment-flow data structure that is readily portable as well as executable by the simulation engine.

An integrated physiological model corresponding to the GI tract of a mammal constructed using STELLA® and the above-described methodology is illustrated in **Figures 24-25**, and **29-39**. An example of information provided by the database is illustrated in **Appendix 4** for the gastrointestinal model depicted in **Figures 24-25** and **29-39**.

A physiologic-based simulation model of the PK tool and method of the invention may optionally include a training/validation model. This aspect of the invention can be used for determining whether the model is specific and accurate with respect to compounds of known membrane transport mechanism (e.g., passive transcellular, passive paracellular, transporter involved for absorption and secretion) and/or with respect to known drug solubility/dissolution rate limitations.

A validation model can be linked to the physiological model of the invention as illustrated in **Figure 11**. The linked system is then run to access the specificity and accuracy computed values for rate and extent of absorption. These values are then compared to empirically measured plasma values. If computed values fall outside of an acceptable range the model can be reevaluated for these compounds and adjustments made to the model.

Data Acquisition:

Input data utilized to generate an absorption profile for a test sample include permeability and solubility parameters, and optionally transport mechanism and dissolution parameters. Input data can be generated de novo following any number of techniques, or obtained from public or existing sources where available. The input data can be derived from chemical, and/or biological assays as well as theoretical predictions. By way of example, the *in vitro* assays may employ artificial (synthetic) or naturally occurring biological preparations. This includes chemical, cell and/or tissue preparations. Assays for generating input data involve screening a plurality of test samples containing isolated compounds and/or isolated mixtures of compounds per test sample in an assay characterized by measurement of (1) permeability and optionally transport mechanism for a test sample; and (2) solubility and optionally dissolution for a test sample. Methods and materials for performing the assays are based on the selected route of administration, the associated barrier(s) to absorption and proposed sampling site(s). For instance, if oral delivery is proposed for simulation and an initial sampling site is selected to be the portal vein (so as to isolate gastrointestinal absorption events from hepatic metabolism) then the input data is collected from an *in vitro* assay that best approximates the luminal barrier and segmental physiology of the gastrointestinal tract.

Examples of some common cell and tissue sources for permeability and transport mechanism assays for a selected route of administration are provided below in **Table 1.**

Table 1: **Permeability and Transport Mechanism.**

Route/Tissue	**Cell Culture**
Oral/Intestinal	Caco-2 cells HT-29 cells T84 cells Intestinal epithelial cells (IEC) SV40 T Immortalized cells Organ culture/co-culture Primary culture
Inhalation/Nasal	SV40 T immortalized cells Primary culture
Ocular/Corneal	RCE1 cells Primary cultures SV40 T immortalized cells
Oral-Buccal/Cheek	Primary cultures
Topical/Transdermal	HaCat cells Primary/co-cultures
IV/Hepatic	Hepatic carcinoma cell lines Primary cultures Co-cultures SV40 T immortalized cells
IV/Blood Brain Barrier	Primary culture SV40 immortalized cells

Examples of some common parameters for solubility and dissolution assays for a given route of administration are provided below in **Table 2**.

Table 2: Solubility and Dissolution Parameters.

Route/Anatomy/Physiology		*In vitro* Parameters
Oral	Gastrointestinal (GI) tract • Stomach • Duodenum • Jejunum • Ileum • Colon	• pH • Temperature • Concentration of test sample • Volume • Osmotic pressure • Admixing conditions • Physiologic Fluid/Buffer/solvent system • Excipients • Other Additives • Test chamber composition
Buccal/Sublingual	Mouth • Cheek • Tongue	
Rectal	Lower GI tract • Colon • Rectum	
Parenteral	Skin Muscles Veins	
Aerosol	Respiratory system • Nose • Lungs • Mouth	
Transdermal	Skin • Topical • Ear	

In vitro and *in vivo* techniques for collecting permeability and transport mechanism data using cell- and/or tissue-based preparation assays are well known in the art (Stewart et al., Pharm. Res. (1995) 12:693-699; Andus et al., Pharm. Res. (1990) 435-451; Minth et al., Eur. J. Cell. Biol. (1992) 57:132-137; Chan et al., DDT 1(11):461-473). For instance, *in vitro* assays characterizing permeability and transport mechanisms include *in vitro* cell-based diffusion experiments and immobilized membrane assays, as well as *in situ* perfusion assays, intestinal ring assays, intubation assays in rodents, rabbits, dogs, non-human primates and the like, assays of brush border membrane vesicles, and everted intestinal sacs or tissue section assays. *In vivo* assays for collecting permeability and transport mechanism data typically are conducted in animal models such as mouse, rat, rabbit, hamster, dog, and monkey to characterize bioavailability of a compound of interest, including distribution, metabolism, elimination and toxicity. For high-throughput screening, cell culture-based *in vitro* assays are preferred. For high-resolution screening and validation, tissue-based *in vitro* and/or mammal-based *in vivo* data are preferred.

Cell culture models are preferred for high-throughput screening, as they allow experiments to be conducted with relatively small amounts of a test sample while maximizing surface area and can be utilized to perform large numbers of experiments on multiple samples simultaneously. Cell models also require fewer experiments since there is no animal variability. An array of different cell lines also can be used to systematically collect complementary input data related to a series of transport barriers (passive paracellular, active paracellular, carrier-mediated influx, carrier-mediated efflux) and metabolic barriers (protease, esterase, cytochrome P450, conjugation enzymes).

Cells and tissue preparations employed in the assays can be obtained from repositories, or from any higher eukaryote, such as rabbit, mouse, rat, dog, cat, monkey, bovine, ovine, porcine, equine, humans and the like. A tissue sample can be derived from any region of the body, taking into consideration ethical issues. The tissue sample can then be adapted or attached to various support devices depending on the intended assay. Alternatively, cells can be cultivated from tissue. This generally involves obtaining a biopsy sample from a target tissue followed by culturing of cells from the biopsy. Cells and tissue also may be derived from sources that have been genetically manipulated, such as by recombinant DNA techniques, that express a desired protein or combination of proteins relevant to a given screening assay. Artificially engineered tissues also can be employed, such as those made using artificial scaffolds/matrices and tissue growth regulators to direct three-dimensional growth and development of cells used to inoculate the scaffolds/matrices.

Epithelial and endothelial cells and tissues that comprise them are employed to assess barriers related to internal and external surfaces of the body. For example, epithelial cells can be obtained for the intestine, lungs, cornea, esophagus, gonads, nasal cavity and the like. Endothelial cells can be obtained from layers that line the blood brain barrier, as well as cavities of the heart and of the blood and lymph vessels, and the serious cavities of the body, originating from the mesoderm.

One of ordinary skill in the art will recognize that cells and tissues can be obtained de novo from a sample of interest, or from existing sources. Public sources include cell and cell line repositories such as the American Type Culture Collection (ATCC), the Belgian Culture Collections of Microorganisms (BCCM), or the German Collection of Microorganisms and Cell Cultures (DSM), among many others. The cells can be cultivated by standard techniques known in the art.

Preferred assays for collecting permeability data utilize devices and methods that measure change in resistance or conductivity of a membrane system by ion flux. Any device suitable for such studies can be employed. These include voltage-clamp type devices and methods that employ either cell cultures or precision tissue slices. Diffusion chamber systems utilizing cultured cells grown on permeable supports to measure permeability are preferred. More preferred devices are readily adapted for high-throughput and automated screening. Examples of such devices are known and exemplified in U.S. Patent No. 5,599,688; WO 96/13721; and WO 97/16717. These devices also can be adapted for examining transport mechanisms. As can be appreciated, however, measurement of resistance, conductivity and/or ion flux is not required to determine permeability of compounds. Many other techniques are available and can be employed in the invention. For instance, permeability data also may be predicted using theoretical models to approximate this parameter, for example, from SAR/QSAR (e.g., log P, molecular weight, H-bonding, surface properties).

Transport mechanism of a test sample of interest can be determined using cell cultures and/or tissue sections following standard techniques. These assays typically involve contacting cells or tissue with a compound of interest and measuring uptake into the cells, or competing for uptake, compared to a known transport-specific substrate. These experiments can be performed at short incubation times, so that kinetic parameters can be measured that will accurately characterize the transporter systems, and minimize the effects of non-saturating passive functions. (Bailey et al., Advanced Drug Delivery Reviews (1996) 22:85-103); Hidalgo et al., Advanced Drug Delivery Reviews (1996) 22:53-66; Andus et al., Pharm. Res. (1990) 7(5):435-451).

For high-throughput analyses, cell suspensions can be employed utilizing an automated method that measures gain or loss of radioactivity or fluorescence and the like such as described in WO 97/49987.

In a preferred embodiment, transport mechanism is determined using high-throughout transporter screening cell lines and assays. In this aspect of the invention a cell line is selected and/or manipulated to over-express one or more transporter proteins, and/or enzymes. The cells are then used to rapidly identify the mechanism(s) by which a compound is transported across the physiological barrier of interest. Transporters of interest represent the basic categories of transport including uptake and efflux transporters. These transporters aid in the movement of materials in biological systems, into and out of cells and across cellular layers. Natural combination(s) of enzyme(s) and transporter(s) also can provide the basis of a high-throughput transport mechanism screening assay. For instance, certain enzymes or transporters require secondary enzymes or transporters to function in a normal physiological mode, i.e., cytochrome P4503A is co-regulated with P-glycoprotein. These proteins share the same substrate and their genes are co-regulated. Thus multiple artificial combination(s) of transporter(s) and enzyme(s) can be employed for characterizing transport mechanism of a test sample of interest. Examples of possible combinations of a transporter and/or enzyme in a host cell of interest include cell-transporter-enzyme, cell-transporter, cell-enzyme, cell-enzyme-enzyme, and cell-transporter-transporter. Examples of transporters that can be used to transfect the host cell of interest include peptide transporters (PepT1), amino acid transporters, organic cation transporters (OCT1), organic anion transporters, nucleotide transporters (N1, N2, N3, ES, EI), glucose transporters (SGLT1, GLUT 1 through GLUT 7), monocarboxylate transporters (MCT1), and multi-drug transporters (LRP, MDR, MRP, PGP). Examples of enzymes that can be used to transfect the host cell are Phase I and II enzymes, cytochrome P450, 3A, 2D and the like.

Nucleic acid and/or amino acid sequences for transporters/enzymes can be identified in various genomic and protein related databases. Examples of publicly accessible databases include GenBank (Benson et al., *Nucleic Acids Res* (1998)26(1):1-7; USA National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, Bethesda, MD, USA), TIGR

Database (The Institute for Genomic Research, Rockville, MD, USA), Protein Data Bank (Brookhaven National Laboratory, USA), and the ExPASy and Swiss-Protein database (Swiss Institute of Bioinformatics, Genève, Switzerland).

Any number of known techniques can be used to prepare nucleic acid encoding a transporter(s) and/or enzyme(s) of interest. To express a target protein in a host cell the nucleotide sequence coding for the polypeptide is inserted into an appropriate expression vector, i.e., a vector that contains the necessary elements for the transcription and translation of the inserted coding sequence. The host cell line can be stably or transiently transfected by methods known in the art. Examples of transient transfection methods include calcium phosphate, electroploration, lipofectamine, and DEAE dextran. A cell line can be stably transfected using methods known in the art such as calcium phosphate. In addition, the host cell can be infected with a retrovirus containing a target protein of interest, resulting in stable expression of the desired target protein. Host cells that express the target gene product can be identified by standard techniques. These include, but are not limited to, detection of the protein as measured by immunoprecipitation and Western blot analysis or by measuring a specific biological response.

For synthesis in a cell, a target transporter/enzyme protein can be generated by standard techniques. Cells that naturally express a target protein can be employed. Transfection and transformation of a host cell with DNA encoding a protein of interest also can be used. For example, a polymerase chain reaction (PCR) based strategy may be used to clone a target DNA sequence encoding all or part of a target membrane polypeptide of interest. (See, e.g., "PCR Cloning Protocols: From Molecular Cloning to Genetic Engineering," B.A. White, ed., Humana Press, Methods in Molecular Biology, Vol. 67, 1997). For example, PCR can be used for cloning through differential and subtractive approaches to cDNA analysis, performing and optimizing long-distance PCR, cloning unknown neighboring DNA, and using PCR to create and screen libraries. PCR also can be used to introduce site-specific and random mutations into DNA encoding a target protein of interest.

For general cloning purposes, complementary and/or degenerate oligonucleotides corresponding to conserved motifs of the target membrane polypeptide may be designed to serve as primers in a cDNA and/or PCR reaction. Templates for primer design can be obtained from any number of sources. For example, sequences, including expressed sequence tags (ESTs) can be obtained from various databases, such as GenBank, TIGR, ExPASy and Swiss-Protein databanks. Homology comparisons performed using any one of a number of alignment readily available programs that employ search engines to find the best primers in a sequence based on various algorithms. Any number of commercially available sequence analysis packages, such as Lasergene, GeneWorks, DNASIS, Gene Jockey II, Gene Construction Kit, MacPlasmap, Plasmid ARTIST, Protein Predictor, DNA/RNA Builder, and Quanta. (See, e.g., "Sequence Data Analysis Guidebook," Simon R. Swindell, ed., Humana Press, 1996). The information can be used to design degenerate primers, nested/multiplex primers, site-directed mutagenesis, restriction enzyme sites etc. Primers can be designed from homology information, and computer programs can be used for primer design as well. Examples include "Primer Premier 4.0" for automatic primer selection (CloneTech, Inc.). The amplified cDNA and/or PCR fragment may be used to isolate full-length clones by radioactive or non-radioactive labeling of the amplified fragment and screening a library.

Alternatively, transporter/enzyme DNA cloned from one source may be utilized to obtain a corresponding DNA sequence from other sources. Specifically, a genomic and/or cDNA library constructed from DNA and/or RNA prepared from a cell known or expected to express the target transporter/enzyme may be used to transform a eukaryotic or prokaryotic host cell that is deficient in the putative gene. Transformation of a recombinant plasmid coding for the protein into a deficient host cell would be expected to provide the cell with a complement product corresponding to the protein of interest. In some cases, a host cell can be selected to express a particular phenotype associated with the target polypeptide and thus may be selected by this property. For a review of cloning strategies which may be used, see e.g., Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual, Cold Springs Harbor Press, New York; and Ausubel et al., 1989, Current Protocols in Molecular Biology, Green Publishing Associates and Wiley Interscience, New York.

To express a target transporter/enzyme in a host cell the nucleotide sequence coding for the protein, or a functional equivalent for modular assembly as described above, is inserted into an appropriate expression vector, i.e., a vector which contains the necessary elements for the transcription and translation of the inserted coding sequence. Host cells containing the coding sequence and that express the target gene product may be identified by standard techniques. For example, these include but are not limited to DNA-DNA or DNA-RNA hybridization; the presence or absence of "marker" gene functions; assessing the level of transcription as measured by the expression of mRNA transcripts in the host cell; and detection of the gene product as measured by immunoassay or by its biological activity.

Once a clone producing the target transporter/enzyme is identified, the clone may be expanded and used to over express the protein(s). If desired, the proteins may be purified using techniques well-known in the art including, but not limited to immunoaffinity purification, chromatographic methods including high performance liquid chromatography or cation exchange chromatography, affinity chromatography based on affinity of the polypeptide for a particular ligand, immunoaffinity purification using antibodies and the like. The purified proteins can then be bound to an artificial membrane matrix and utilized for assessing interaction of compounds to the transporter/enzyme of interest.

Some commonly used host cell systems for expression of transport proteins and enzymes include *E. coli*, Xenopus oocytes, baculovirus, vaccinia, and yeast, as well as many higher eukaryotes including transgenic cells in culture and in whole animals and plants. (See, e.g., G.W. Gould, "Membrane Protein Expression Systems: A User's Guide," Portland Press, 1994, Rocky S. Tuan, ed.; and "Recombinant Gene Expression Protocols," Humana Press, 1996). For example, yeast expression systems are well known and can be used to express and recover target transporter/enzyme systems of interest following standard protocols. (See, e.g., Nekrasova et al, *Eur. J. Biochem.* (1996) *238*:28-37; Gene Expression Technology Methods in Enzymology 185: (1990); Molecular Biology and Genetic Engineering of Yeasts CRC Press, Inc. (1992); Herescovics et al., FASEB (1993) 7:540-550; Larriba, G. *Yeast* (1993) *9*:441-463; Buckholz, R.G., *Curr Opinion Biotech* (1993) *4*:538-542; Mackett, M, "Expression of Membrane Proteins in Yeast Membrane Protein Expression Systems: A Users Guide," pp. 177-218, Portland Press, (1995).

For high-resolution screening and validation, tissue-based assays may be employed to characterize transport mechanisms. For example, of the cytochrome P450 superfamily, CYP3A enzymes represent the most abundant isoforms in the liver and they are responsible for the metabolism of compounds of diverse chemical structure. The uptake of a compound into hepatocytes can be mediated by passive or carrier processes. Once in the parenchymal cell of the liver, the drug can be metabolized or bind to intracellular proteins. The drug or its metabolite(s) may return to the circulation or exit from the hepatocyte into the bile canaliculus, again by passive or carrier-mediated transport, before secretion in bile. Experimental systems have been devised to study these processes in isolation. Examples of such systems include isolated perfused rat liver (IPRL), and bile duct cannulated (BDC) rat models. (Chan et al., DDT (1996) 1:461-473).

Tissue from transgenic animals designed to express particular transport properties in one or more particular tissues also may be utilized to characterize transport mechanisms. In this aspect of the invention, an animal can be genetically manipulated to express or not express one or more specific proteins in a tissue of interest, e.g. transporter protein in duodenum tissue. Tissue from the genetically engineered animal can then be used to examine transport mechanisms in a tissue-based assay. Transgenic animal methodologies are well known (Gordon et al., Hum. Cell (1993) 6(3):161-169; and Jaenisch, R., Science (1998) 240:1468-1474).

Artificially engineered tissue also can be used for permeability assays, such as tissues generated *ex vivo* for use as skin grafts, transplants, and the like. Such tissues can be obtained using standard techniques. See, for example, U.S. Patent Nos. 5,759,830; 5,770,193; and 5,770,417.

Solubility and dissolution data can be obtained in an *in vitro* assay by testing each sample of interest in an appropriate physiologic fluid/buffer system that best approximates the particular physiological system selected as the barrier to absorption. A solubility profile is a plot of solubility of a test sample at various physiological conditions. As an example, the natural pH environment of the gastrointestinal tract varies from acidic in the stomach to slightly alkaline in the small intestine and fluid composition for each segment may vary as well. The solubility profile provides an estimation of the completeness of dissolution of a test sample in a particular physiological compartment or anatomical entity. In this instance, a panel of test wells each having different pHs and physiological fluid composition can be employed to generate a solubility profile for each test sample. Solubility and dissolution data can also be predicted using theoretical models to approximate these values, for example, from SAR/QSAR information.

In vitro dissolution assays measure the rate and extent of dissolution of a test sample in an aqueous solution. Various parameters are considered when performing a dissolution assay and are well known in the art. These parameters include size of the experimental vessel, amount of agitation and nature of the stirrer, temperature and nature of the dissolution medium, pH, viscosity, and design of the dissolution apparatus. Standard methods known in the art for measuring dissolution include rotating basket, paddle, rotating bottle, flow-through dissolution, intrinsic dissolution, and peristalsis methods. These methods can be adapted and used as a guide for high-throughput solubility and dissolution testing.

For high-throughput collection of solubility and dissolution data, automated methods of solid and liquid handling are employed. This method involves addition of samples to a multi-well or multi-tube/plate system. The data associated with these tubes/plates, such as physiologic fluid/buffer system, volume, concentration, pH and tube/plate maps, is transferred into an inventory system. The inventory system generates codes containing updated information pertaining to the aliquoting, diluting, or pooling methods applied to the original tubes/plates. Tasks created in the database are then carried out physically in coded tubes/plates. Aliquots are then distributed to designated screen sites. After testing, the solubility profiles are generated and ported to a database for access and analysis.

Properties in addition to absorption that can be utilized as input into the PK tool and method of the invention when adapted with the appropriate compartments include metabolism, distribution, and elimination, and optionally toxicity. As with absorption, assays to characterize the relevant data are based on the selected route of administration. Metabolism or biotransformation refers to the biochemical transformation of a compound to another chemical form. The biotransformation process typically results in a metabolite that is more polar (water-soluble) than the original parent molecule.

Most tissues have some metabolizing capacity but the liver is by far the most important organ, on the basis of size if not always concentration of target compound metabolizing enzyme. Phase I reactions are defined as those that introduce a functional group to the molecule and phase II reactions are those that conjugate those function groups with endogenous moieties.

Since metabolism is a drug clearance process, metabolism of a compound contributes to elimination of the compound. Thus, compounds can be tested for metabolism in order to generate input data that considers disposition of a test compound after or concurrent with administration using standard techniques known in the art. (See, e.g., Sakuma & Kamataki, Drug metabolism research in the development of innovative drugs, In: Drug News & Perspectives (1994) 7 (2):82-86).

Metabolism assays for high-throughput screening preferably are cell-based (cells and cellular preparations), whereas high resolution screening can employ both cell and tissue-based assays. In particular, test samples from compound libraries can be screened in cell and tissue preparations derived from various species and organs. Although liver is the most frequently used source of cells and tissue, other human and non-human organs, including kidney, skin, intestines, lung, and blood, are available and can be used to assess extra-hepatic metabolism. Examples of cell and tissue preparations include subcellular fractions (e.g., liver S9 and microsomes), hepatocytes (e.g., collagenase perfusion, suspended, cultured), renal proximal tubules and papillary cells, re-aggregate brain cells, bone marrow cell cultures, blood cells, cardiomyocytes, and established cell lines as well as precision-cut tissue slices.

Examples of *in vitro* metabolism assays suitable for high-throughput screening include assays characterized by cytochrome P450 form-specific metabolism. These involve assaying a test compound by P450 induction and/or competition studies with form-specific competing substrates (e.g., P450 inhibitors), such as P450 enzymes CYP1A, 3A, 2A6, 2C9, 2C19, 2D6, and 2E1. Cells expressing single or combinations of these or other metabolizing enzymes also may be used alone or in combination with cell-based permeability assays. A high-throughput cell-based metabolism assay can include cytochrome P450 induction screens, other metabolism marker enzymes and the like, such as with measurement of DNA or protein levels.

Suitable cells for metabolism assays include hepatocytes in primary culture. Computer-implemented systems for predicting metabolism also may be employed.

For distribution and elimination data, *in vitro* assays can be performed to assess protein binding to a test compound, since protein binding can affect compound distribution and elimination. In general, it is free compound that diffuses into cells and tissues. Binding can be classified as restrictive or permissive with regard to elimination, or quantitatively defined in terms of affinity. Affinity of the binding is defined as low or high when reversible, or more unusually when irreversible binding occurs. The biological half-life of a test compound will increase due to its interaction with a protein. Usually, the higher the affinity the lower the elimination that may be observed. Albumin is by far the most frequent contributors to plasma protein binding since it comprises about one half of the total plasma proteins. The a1-Acid glycoprotein also plays an important role in the protein binding of a compound since it has an affinity for bases (many drugs are weak bases). It is an acute phase reactant and its concentration rises in inflammatory processes, malignant disease and stress. Lipoproteins (HDL, LDL or VLDL) bind drugs that are highly liposoluble and a fairly specific ligand-protein interaction occurs between certain steroids and gamma globulins. Thus, *in vitro* protein binding assays that employ one or more of albumin, a1-acid glycoprotein, lipoprotein, steroid and gamma globulins may be utilized to collect distribution and elimination data that can be utilized for further data collection.

Similarly, toxicity of a test compound may also be assayed and used to generate relevant toxicity data for a test compund. Any number of techniques in the art may be employed for this purpose. Preferred methods are *in vitro*. Examples include determination of toxicity mechanisms, determination of cytotoxic potentials in cell and tissues of target organs, estimation of therapeutic indices from *in vitro* data, cytotoxicity screening of closely related drug compounds in cells from the same mammal or from different species, detection and quantification of peroxisome proliferation, screening of agents to prevent or reverse cytotoxicity, and specialized studies on target cells using co-incubation systems, e.g., red blood cells and hepatocytes.

Toxicity assays may utilize any technique that provides a toxicity parameter as an endpoint. For high-throughput screening, cell based assays are preferred. This includes gene expression (e.g., protein or nucleic acid based) enzymatic activity, and morphology screens and the like. Examples of cell-based assays include *in vitro* peroxisome proliferation studies, which can be used to assay palmitoyl CoA-oxidation in primary hepatocyte culture, with or without concurrent measurement of DNA or protein levels. Cytotoxicity assays in primary cultures also can be utilized, and include screening for cytotoxicity in hepatocytes or renal proximal tubules, enzyme release (lactate dehydrogenase), and MTT conversion (mitochondrial function) following standard techniques. Computer-implemented SAR/QSAR models for predicting toxicity also may be employed, such as when structural information is available.

PK Tool and System Structure:

The PK tool and system of the invention has the structure shown in **Figure 4**. The I/O system provides the user's inputs to the simulation model of the mammalian system of interest. The simulation engine in turn computes one or more of the bioavailability parameters of the compound in the context of one or more physiologic-based segments of the mammalian system under investigation. The output of the simulation engine is then provided to the I/O system.

Operations of the PK tool and system are exemplified in **Figures 3** and **44-46**. After start, the first block is the I/O block (1), where the user enters the inputs and outputs to the system. The I/O system includes I/O panels, for example graphical user interfaces. This may include sub-panels depending on the selected model (see, e.g. **Figure 47**). The I/O system may optionally include one or more databases of simulation models and/or parameters for a given simulation model that the user may access as illustrated in **Figure 45**. The PK tool and system starts with the user inputs and then computes and displays the results in the output space. The input and output space can be selected, e.g., by toggling, or by a menu. It is to be understood that on-line helps also are available to give a user information, and to guide the user through the PK tool and system user interface.

For input, the Menu function presents various choices to the user. These choices include dose, permeability, and solubility among others. The user then enters the relevant values corresponding to a given physiological segment of the selected mammalian system in question. Depending on the simulation model that the user chooses, the Menu function will provide options for data input, such as pH, transit time, run time, and formulation release rate.

The Menu function also presents various choices to the user after the results for a simulation have been obtained. The choices open to the user include one or more of the functions "Rate of Absorption," "Extent of Absorption," "Concentration," "Print Graph," "Print Table," and "Quit" among others.

For predicting absorption parameters, input of the data is the first operation that the PK tool and system of the invention performs when activated. In this operation, the user enters the appropriate value of each input variable into the input panel in a form readable or convertible by the system to a readable form and obtains complete results in the output panel. Alternatively, the PK tool and system can be adapted to receive structural information that the system, or a separate interfaced system converts to the relevant input parameter values. For this function the user inputs the compound structure in a form readable or convertible by the system to a readable form. This includes standard chemical formulas, chemical names, SMILE strings, as well as two-dimensional and/or three-dimensional structures.

After the user inputs the initial data, the Start Simulation function is selected. In the simulation function, the simulation engine is activated. The user may then choose to invoke the Stop Simulation function, to terminate the simulation, or allow the simulation engine to proceed with until a user specified or system default time point is reached. The user may then view, print, save and/or export then results using output functions, including printing of the I/O panel. This includes numerical, tabular, and graphical formats. These options are selected by the user through the Menu function.

The Quit function exits the PK tool and system. One aspect of the output functions and the Quit function is to save the generated information in a format that allows them to be an input to other programs, such as the SAR or QSAR CAD program.

Forward Mode Operation of the PK Tool:

In the forward mode operation mode, the user enters the input data, and the PK tool reacts as described above. In one embodiment, the PK tool displays a numerical representation or graphic of the test compound or selected PK profile thereof. Also displayed are parameters that can effect fate of the compound in one or more compartments of the mammal, e.g., the dose, formulation, pH, fluid volume, fluid absorption (fluid secretion), dissolution rate, cumulative dissolution, transit, pH-dependent solubility and dissolution and the like. Other variables may also be available, e.g., through a data box.

The forward mode operation of the simulation engine displays the resulting PK parameters, such as absorption.. Changing any parameter causes recalculation of the PK quantities, invoking the the simulation engine and its associated simulation model. The forward mode operation provides either, or both, a display or a printout of the PK parameters for a test compound.

Backward Mode Operation of the PK Tool:

In backward mode operation of the PK tool, the user is allowed to assess formulations for a compound. In this aspect of the invention, the user specifies the required absorption profile, or absorption parameter for a compound. The tool then generates the formulation release rates for the compound that meets the requirements. The user can then compare the solution set, against previously qualified compounds and formulation designs drawn from a database and new, unqualified designs created by the tool and method of the invention.

Predictability:

The PK tool and method of the invention permit a high level of accuracy in predicting bioavailability of molecules from the following four classes of compounds: a) passive transcellular; b) passive paracellular; c) transcellular transporter involved; d) apically recycled. The evaluation is based on the difference between bioavailability values predicted by the model and known bioavailability values. For example, conformation of predictability for human GI absorption values for passive transcellular molecules is evaluated with dissolution rate limitations and solubility limitations. If the computed values fall outside of an acceptable range ($r^2 > 0.75$ predictability), the PK model is reevaluated for these compounds and adjustments made to the model. Similarly, absorption measures that deviate from known values are reevaluated and appropriate modifications made to the model (e.g. iterative process).

The PK model can be used to predict bioavailability in a mammal using dose (actual or estimated) and various input data. Examples include (1) permeability data alone; (2) permeability data together with solubility and dissolution data; (3) permeability data together with animal data; and/or (4) permeability, animal and human clinical data. Validation of the model is defined as follows, where greater than 80% of the compounds tested will fall within the following prediction criteria.

1. Predictability of the PK tool using permeability data alone with limits for dose and elimination rate ($r^2 > 0.75$ predictability).

2. Predictability of the PK tool using permeability and solubility data along with limits for dose and elimination rate ($r^2 > 0.75$ predictability).

3. Predictability of the PK tool using permeability data together with animal data for pharmacokinetics together with limits for dose ($r^2 > 0.85$ predictability).

4. Predictability of the PK tool using permeability and animal or human IV data to predict absorption values for molecules with solubility limitations ($r^2 > 0.85$ predictability).

The correlation coefficient can be calculated using data from the predicted line from pharmacokinetic fitting as the observed data points and as the predicted fit, and the output of physiologic-based simulation model coupled to the systemic kinetics for that compound. The prediction power of a given physiological simulation model can be demonstrated by simulating the plasma levels in compounds. Other methods can be utilized to assess the predictive power of the model to achieve the same end result (i.e., evaluation of model performance).

The method and PK tool of the invention allows the drug developer to go from a set of user inputs, to predicting the fate of the compound in a mammalian system of interest, to selection of a compound design input to a SAR or QSAR CAD tool, and to chemical synthesis development, validation and high level drug development. The PK tool and system may advantageously be interfaced with other databases and/or systems. For example, the system may be built around an expert system-database manager path. The menu can invoke the on-line documentation, the database, and any member of the expert system-database system.

The PK tool and method of the invention can be used to predict the rate and extent of absorption of compounds as well as regional concentrations relative to one or more selected sampling sites across a physiological barrier of a mammalian system of interest. The PK tool and method of the invention also can be used in combination with prediction of additional bioavailability parameters such as distribution, metabolism and elimination, as well as toxicity. Thus this information can be used to supplement and significantly reduce animal testing during pre-clinical testing. The PK tool and method of the invention also are particularly useful for screening compounds earlier in the drug discovery process. For instance, the PK tool and method may be employed in the screening and ranking of compounds before, during and/or after receptor activity testing, thus increasing the odds of selecting a lead compound that will survive clinical studies, resulting in decreased development costs, faster approval time, and consequent lower drug prices. This permits selection and ranking of lead compounds that not only have optimal receptor activity, but also exhibit optimal bioavailability.

The following Examples are intended to illustrate various aspects of the invention and are not intended to limit the scope of the invention.

EXAMPLES

Example 1: Introduction to Model Design and Development

A physiologic-based simulation model for predicting oral absorption of a compound in a mammal from *in vitro* (e.g., tissue, cell and SAR/QSAR) and *in vivo* data (e.g., human) was constructed in two primary stages. The first stage involved development of a mass-based multi-compartment simulation model (mass model), a volume-based multi-compartment simulation model (volume model) and an integrated mass-volume multi-compartment simulation model (mass-volume model). These models were individually tested and validated for five segments of the GI tract: the stomach, the duodenum, the jejunum, the ileum, and the colon. The second stage involved development of an integrated multi-compartment physiological model of the GI tract (GI model). The models were developed using a combination of *in vitro* data and *in vivo* data.

A computer-based mathematical model development tool with a graphical user interface was employed to design and construct the initial simulation models. The computer program STELLA® was selected as suitable for this purpose since it permitted compartment model building and mathematical equation modification and at each stage of the build, as well as calculation of flow between compartments at user-specified time intervals (dt) with user-specified input functions and values. An example of iconic tools and description, as well as graphically depicted compartment-flow models generated using STELLA® and their relation to a conventional pharmacokinetic IV model is illustrated in **Figures 5-8**.

Example 2: Compound Data Sets

Compound data sets for development, and thus building, testing, training and validation of the models were obtained from various sources including the literature and cell, tissue, animal and human tests as described herein. The data sets included relevant physiological parameters related to absorption of a compound including GI tract related parameters (e.g., pH, initial volumes, surface area, average transit time, volume transfer rates, new water absorption etc.) and physicochemical compound related parameters (e.g., dissolution, permeability, solubility etc.).

Data sets were selected for compounds that permitted development and isolated testing and validation for each stage of the build. Compounds suitable for this purpose were chosen as follows. For the mass, volume and integrated mass-volume simulation models, a candidate compound was chosen based on the premise that the best candidate compound for model development would not be a drug that is highly correlated pharmacokinetically between cell, tissue, animal and humans, but one that is poorly correlated. That is, a compound predicted to have high total absorption in humans based on pre-clinical studies, but ultimately exhibited poor absorption in humans when tested in clinical trials was chosen. Additionally, a compound was selected that is not subject to pre-absorptive or hepatic metabolism so as to isolate absorption components of the models from pre-absorptive and metabolic factors. Gancyclovir (9-(1,3-dihydroxy-2-propoxymethyl)guanine, monosodium salt (DHPG) or Cytovene) was suitable for this purpose. Also, significant animal and human clinical data was publicly available for Gancyclovir (Jacobson *et al.*, *Antimicrobial Agents and Chemotherapy*, Vol. 31, No. 8, p. 1251-1254 (1987); New Drug Application for Gancyclovir Sodium (Syntex, Inc. USA), obtained from the Food & Drug Administration; Drew *et al.*, *New England Journal of Medicine*, (1995) 333:615-610; and Anderson *et al.*, *Clinical Therapeutics*, (1995) 17:425-432 (1995)).

For development and testing of the integrated GI model, a set of training and testing lead drug compounds in various stages of human clinical testing were selected. This test set included compounds having diverse dosage requirements and ranges of permeability, solubility, dissolution and transport mechanisms, as shown below in **Table 3**.

Table 3.

Compound Test Set				
Compound	Permeability	Solubility	Dose	Mechanism of Absorption
α1	++++	++++	+++++	active
α2	++	+++	+++++	paracellular
α3	+	+	++++	unclassified
α4	+	++++	++	transcellular
α5	+	+++	++++	paracellular
α6	++++	++	++++	transcellular
α10	++++	+++++	+	transcellular
β1	+++++	+++++	+	transcellular
β2	++++	++	++	transcellular
β3	+	+	+++	paracellular
β5	++++	++	+++	unclassified
β6	+	+++++	+++	unclassified

+++++ = greatest value & + = lowest value

Example 3: Experimental Data Collection and Processing

Experimentally derived *in vivo* and *in vitro* data was obtained as follows. To ensure quality data was used for training and validation, experimental conditions were specific enough to ensure proper data collection techniques, but flexible to allow minor and insignificant variations in individual protocols. Data sets used for model development included individual data points, i.e., raw data, that was analyzed and processed by stepwise regression analysis using a least squares minimization technique or similar fitting tool. In particular, data processing for permeability involved separation of compounds by absorption mechanism and into training and validation sets. pH dependent solubility profiles were interpolated to obtain complete profiles. For dissolution, data points were fit to determine dissolution rates. For human clinical data, data analysis and processing employed a pharmacokinetic IV/PO model and weighted least-squares regression analysis (See **Figure 18**). The IV/PO model includes a central compartment in equilibrium with a peripheral compartment, a pre-systemic compartment re-circulated with the central compartment and for input

PO doses (error function input), a hepatic compartment, as well as an IV dose and first-order elimination compartment. The plasma sample is taken from the central compartment, and the FDp sample from the hepatic compartment.

A. Human *In vivo* Data – Oral (PO)

Plasma levels following oral administration (PO) in humans were used to determine the amount of compound input to the hepatic vein (FDp) as a function of time. Plasma levels of drug in humans following oral administration of drug solution or suspension after an overnight fast were used as a data set. If no solutions or suspensions were administered, formulated dosage form data were used. The PO profiles included individual data points for each patient enrolled in the study from the time of administration through 24 hours to 32 hours after administration, along with dosage. If multiple dose regimens were administered, plasma profiles for all doses were used.

B. Human *In vivo* Data – Intravenous Administration (IV)

Plasma levels following intravenous administration (IV) in humans were used to determine the amount of drug input to the hepatic vein (FDp) as a function of time. IV profiles included individual data points for each patient enrolled in the study from the time of administration through 24 hours to 32 hours after administration, along with the dose. If multiple dosage regimens were administered, plasma profiles for all doses were used.

C. *In vitro* Permeability Data

In vitro permeability data was used to calculate drug fluxes across various regions of the intestinal mucosa. This included rabbit intestinal tissue from one or more of duodenum, jejunum, ileum and colon, and Caco-2 cells. The mechanism of transport, such as passive transcellular or paracellular, carrier-mediated absorption, carrier-mediated secretion, or mixed mechanism, was determined for several test compounds and permeabilities for each mechanism and assessed as listed in **Table 4**. Protocols for permeability assays are described in **Example 4**.

Table 4: Transport mechanism permeabilities and parameters for each GI region.

Mechanism	Permeabilities	Parameters
Passive transcellular	Apical to basolateral (AP to BL)	P_c
Passive paracellular	AP to BL	P_e
Carrier-mediated absorption	AP to BL without inhibition	K_m, P_c, and P_m, or P_e at entire concentration range
Carrier-mediated secretion	AP to BL and BL to AP without inhibition	P_m, P_c, and P_m, or P_e at entire concentration range

D. Solubility Data

Solubilities of test compounds as a function of pH were determined from pH 1.5 to 8.2 in increments of 0.1 pH units. Protocols describing conditions for solubility determination are found in **Example 4**. Alternatively, solubility at each pH unit from 1.5 to 8.0 was used, with a minimum of 5 data points at pH 1.5, 6.0, 6.5, 7.0, and 7.5. These solubilities were used to calculate the amount of soluble compound available for absorption across the intestinal mucosal barrier.

E. Dissolution Data

The dissolution of test compounds as a function of pH were determined at pH 1.5, 6.0, 6.5, 7.0, and 7.5. Protocols describing conditions for dissolution determination are found **Example 4**. The dissolution of powdered compound, and alternatively, dissolution/disintegration data for the formulated dosage form used to collect oral plasma profiles were used. The dissolution data were used with solubility data to calculate the amount of drug available for absorption across the intestinal mucous within each region of the intestine.

Example 4: Protocols for Data Collection

Provided below are detailed protocols utilized for collecting and calculating data described in **Example 3**. These protocols were employed to ensure the quality of the data provided for development of the simulation models.

A. *In vitro* Permeability Protocols

1. Diffusion Chambers

Permeability data is determined using intestinal tissue in vertical diffusion chambers similar in design to NaviCyte 8X24 mm, 9mm Low-volume, or 9mm round tissue diffusion chambers. The chamber system used maintains the tissue as well as the donor and receiver buffers at 37°C. Both the donor and receiver buffers within the chamber are continuously mixed throughout the experiment.

2. Mathematical Calculations

Effective permeability (Pe) is calculated using **Equation 2**.

$$P_e = \frac{V}{AC_0} \cdot \frac{dC}{dt} \qquad \text{(Eq. 2)}$$

where V is the volume of the receiver chamber (ml), A is the surface area available for diffusion (1.78 cm2 for 8X24 mm chambers, 0.64 cm2 for 9 mm round and Low-volume chambers), C_0 is the donor concentration, and dC/dt is calculated as the slope of the regression line of the corrected receiver concentration (see Sampling) v. time plot. Two conditions must be satisfied for this equation to apply: (1) sink conditions in the receiver chamber, i.e. the accumulated concentration, must be virtually zero when compared to the donor concentration; and (2) the donor concentration must be constant (C_0) throughout the experiment.

The parameters for carrier-mediated absorption and secretion are calculated using **Equation 3**.

$$P_e = \frac{P_c}{1 + \frac{C_0}{K_m}} + P_m \qquad \text{(Eq. 3)}$$

where Pc is the carrier-mediated permeability, Pm is the passive permeability, Km is the affinity of the drug for the carrier, and C_0 is the donor concentration. Pc, Pm, and Km are calculated using non-linear regression, Pe is calculated using **Equation 2**, and C_0 is given as part of the experimental conditions. To obtain valid parameter values, Pe is determined for a sufficient number of C_0's to determine Km using **Equation 3** (a minimum of 6 C_0's is recommended ranging between the analytical limit and the solubility limit). If Pe values are provided, the variability of the mean as well as the number of experiments performed for each concentration are provided to allow accurate regression analysis.

3. Experimental Conditions

a. Buffers

Experiments are performed in appropriate, non-cytotoxic, physiological saline iso-osmotic buffers at pH 7.4 (basolateral/serosal side) or pH 6.5 (apical/mucosal side). Preferred buffers are Ringer's buffer (pH 7.4), Ringer's with glucose (pH 7.4), MES ringer's buffers (pH 6.5), or MES Ringer's with glucose (pH 6.5) (**Table 5**).

Table 5: Formulas for Ringer's buffer and Ringer's with glucose buffer.

Chemical	Ringer's buffer (mM)	Ringer's with glucose (mM)	MES Ringers Buffer (mM)	MES Ringer's With glucose (mM)
KCI	5	5	5	5
Na_2HPO_4	1.15	1.15	--	--
Na_2HPO_4	0.3	0.3	--	--
$NaHCO_3$	25	25	--	--
$MgSO_4$	1.1	1.1	1.1	1.1
$CaSO_4$	1.25	1.25	1.25	1.25
NaCI	qs iso-osmotic	qs iso-osmotic	qs iso-osmotic	qs iso-osmotic
MES	--	--	25	25
Glucose	--	25	--	25

pH adjusted with 1 N HCI or 1 N NaOH

b. Sampling

Samples are collected from the receiver chamber beginning once steady state has been achieved and continuing for at least 90 minutes. Four to six (preferred) samples are collected to allow accurate determination of dC/dt (**Equation 2**). The volume removed from the receiver chamber at each time point is replaced with buffer containing no drug to maintain constant volume in the receiver chamber. The dilution of the receiver concentration due to the addition of buffer is corrected during data analysis and Pe calculation. The concentration may be corrected by: (1) adding the mass removed at each sampling time to the mass removed from the receiver chamber at all prior sampling times, by summing calculated mass absorbed and adding to mass for sample calculation; and (2) using **Equation 4** (preferred).

$$\frac{1}{X} = -\sum_{n}^{k} (-1)n \frac{\beta}{n} \left(\frac{(S)}{(V)}\right)^{n-1}$$

(Eq. 4)

where the corrected receiver chamber concentration is obtained by dividing the collected sample concentration by **Equation 4** (1/X), S is the volume of sample withdrawn, V is the receiver chamber volume, k is the sequential sample number, i.e., k=1 for the first sample time, k=2 for the second sample time, k-3 for the third sample time, etc., and β is the corresponding number from Pascal's triangle (**Table 6**).

Table 6: Pascal's Triangle for determining β coefficients.

Sample	1st term	2nd term	3rd term	4th term	5th term	6th term
1	1					
2	1	1				
3	1	2	1			
4	1	3	3	1		
5	1	4	6	4	1	
6	1	5	10	10	5	1

Donor concentration (C_0) is determined by sampling the donor buffer containing the test compound with subsequent analysis directly from the donor chamber, or from a stock solution of donor buffer provided binding and absorption to the interior of the chambers does not occur.

c. Intestinal Tissue

Rabbit intestinal tissue is used for permeability experiments. During mounting of tissue onto chambers, intestinal muscles are stripped off the mucosa and discarded. Care should be taken to ensure integrity of the tissue. A minimum of three chambers are used to determine P_e values for each region, concentration and compound. The mean P_e and Standard Error of the Mean are provided for each study.

d. Cell monolayers

Caco-2 cell monolayer Pe is determined in diffusion chambers similar to NaviCyte Snapwell™ diffusion chambers and follow all procedures described above except the recommended buffers are Ringer's with glucose or MES Ringer's with glucose as listed in **Table 6**.

Caco-2 cells are grown using DMEM media supplemented with 10% FBS, 5% PCN-STEP, and 1% NEAA under 95-100% humidity and 5% CO_2 at 37°C. Cells are grown in flasks and the culture split at 85-95% confluence. Snapwells™ are seeded at 65,000 cell/cm^2 and used in the permeability experiment within 21-28 days post seeding to allow for differentiation.

4. Determination of absorption mechanism

Absorption mechanism for a compound is determined by one of the following methods. Determination of P_e in both the apical-basal (AB) to basal-lateral (BL) and BL to AB directions using **Equation 2**, or determination of P_e in the AB to BL direction at concentrations, (a) close to the analytical limit, and (b) close to the solubility limit.

Similar P_e values in both the AB to BL and BL to AB indicate a passively absorbed compound and no further studies are required. AB to BL P_e greater than BL to AB indicates carrier-mediated absorption and P_e must be determined for 5 additional C_0 in the AB to BL direction. BL to AB P_e greater than AB to BL indicates carrier mediated secretion and P_e determined for 5 additional C_0's in the BL to AB direction.

Similar P_e values at low and high concentrations indicate a passively absorbed compound, and no further studies are required. Low concentration P_e higher than high concentration P_e indicates carrier-mediated absorption and Pe is determined for 5 additional C_0's in the AB to BL direction. High concentration P_e higher than low concentration P_e may indicate carrier-mediated secretion. BL to AB P_e is then determined at the low concentration and the mechanism determined as described above.

B. Solubility determination

Solubility of a compound is determined using an accurate and scientifically sound method similar to the Phase Rule and Phase-solubility analysis as described in Remington's: The Science and Practice of Pharmacy, 19th edition, Chapter 16.

The solubility is determined at pH 1.5 using Simulated Gastric Fluid (USP XXII) minus pepsin. Solubility at pH 6.0, 6.5, 7.0, and 7.5 is determined in Simulated Intestinal Fluid (USB XXII) minus pancreatin. Parameters are for data collection are carefully monitored by ensuring purity of the test compound and accuracy of the Simulated Gastrointestinal fluids. A temperature of 37°C is maintained accurately during the course of the determination. Complete saturation and accurate analysis of saturated solutions are employed.

C. Dissolution determination

The dissolution rates are determined using the equipment, apparatus, and methods described in USP XXII, <711> dissolution. The dissolution rate at pH 1.5 is determined in Simulated Gastric Fluid (USP XXII) minus pancreatin. Concentrations are collected and analyzed for drug compound from the vessel for a sufficient time (6 hours, preferable) to allow the initial slope of the concentration v. time curve to be determined. The slope (dissolution rate) is determined using the initial linear portion of the concentration v. time plot if non-sink conditions exist. Under sink conditions, the entire plot are used to calculate the slope. The slope is reported as the dissolution rate. Explanations of the dissolution rate, sink and non-sink conditions, and equations for calculation are given in Remington's: the Science and Practice of Pharmacy, 19th edition, Chapter 34.

If a formulated dosage form is used for dissolution testing, the dissolution protocols described are used to determine the dissolution rate for drug compound from the formulated dosage form.

Example 5: Standards and Protocols for Evaluating Permeability Data Collection

This example provides detailed protocols for controlling the quality of permeability data collection described in **Examples 3** and **4**. Compounds listed in **Table 7** are used as standards for monitoring permeability data collection and quality. The compounds were chosen to represent each intestinal transport mechanism (passive transcellular, passive paracellular, carrier-mediate influx, or carrier-mediated efflux).

Table 7: Permeability Standards

Transport mechanism	Compounds
Passive Paracellular	mannitol
Passive Transcellular	hydrocortisone
Carrier-mediated Influx	D-glucose
Carrier-mediated Efflux	etoposide

Mannitol, hydrocortisone, D-glucose, and etoposide also were chosen since they are widely used as markers for intestinal transport across rabbit tissue and other systems with well characterized Pe values. These compounds also are available commercially as either 3H-labeled or 14C-labeled.

Permeability data for standards is compared to the values for rabbit listed in **Table 8** (or other standard values) using basic statistical analyses. If the data is significantly different ($p\text{-value}>0.05$) for any of the standard compounds, data collection is repeated.

Table 8: Transport Characteristics of Permeability Standards*

Compound (donor concentration)	Pe (cm/s)			
	Duodenum	Jejunum	Ileum	Colon
mannitol (1mM)5	1.73×10^{-6}	3.54×10^{-6}	4.02×10^{-6}	5.53×10^{-6}
hydrocortisone (0.01 μM)5	3.00×10^{-7}	1.31×10^{-6}	2.91×10^{-6}	3.85×10^{-6}
D-glucose (10 mM)5	4.55×10^{-6}	1.02×10^{-5}	1.45×10^{-5}	9.28×10^{-6}
etoposide (100 μM)				

*Note: permeability values are representative of ranges. Other values or extended ranges may be used.

A. Experimental Conditions

Protocols, conditions and calculations for permeability evaluation of standards are as described in **Example 4**, with the following modifications.

Permeability experiments are performed using Ringer's buffer at pH 7.4 on both the apical/mucosal and basolateral/serosal sides. Ringer's buffer is as described above excepting that glucose is substituted with mannitol when Pe values for glucose are being measured.

Samples are collected from the receiver chamber beginning 30 minutes after experiment initiation and continuing every 15 minutes until 6 samples have been collected (105 minutes). One-half ml is removed from each receiver chamber at each time point and compound concentration determined. The volume removed from the receiver chamber is replaced with buffer containing no drug to maintain constant volume in the receiver chamber. The dilution of the receiver concentration due to the addition of buffer should be corrected during data analysis and Pe calculation. The concentration is corrected by using **Equation 5**.

$$\frac{1}{X} = \sum_{n=1}^{k} (-1)^{n-1} \frac{\beta}{k+1} \left(\frac{S}{V} \right)^{n-1} \qquad \text{(Eq. 5)}$$

Where the corrected receiver chamber concentration is obtained by dividing the collected sample concentration by **Equation 5** (1/X), S is the volume of sample withdrawn, V is the receiver chamber volume, k is the sequential sample number, i.e. k=1 for the first sample time, k=2 for the second sample time, k=3 for the third sample time, etc., and β is the corresponding number from the modified Pascal's triangle below (**Table 9**). Note: Since the sample intervals are not even (i.e. the 1st interval is 30 minutes, all others 15 minutes) **Equation 5** as well as the β coefficients are modified from those listed in **Example 4.**

Table 9: Modified Pascal's Triangle for determing β coefficients

Sample	1st term	2nd term	3rd term	4th term	5th term	6th term
1	2					
2	3	2				
3	4	5	2			
4	5	9	7	2		
5	6	14	16	9	2	
6	7	20	30	27	11	2

The donor concentration C_0 is determined by sampling 0.02 ml of the donor buffer containing drug (with subsequent analysis) directly from the donor chamber. Potential binding of drugs to the chambers also is monitored. Donor samples (0.02 ml) are taken at experiment initiation and at experiment conclusion. If a significant decrease in drug concentration has occurred (>10%) the experiment is repeated using procedures which compensate for the drug loss in the donor chamber. It is recommended that the donor chamber solution be removed and replaced with fresh donor buffer containing drug at appropriate intervals. The intervals and volumes to be used are determined using sound scientific judgment. Adequate data is collected to show the donor drug concentration has remained constant throughout the experiment.

For tissue-based permeability assays, during mounting of tissue onto chambers, intestinal muscles should be stripped off the mucosa and discarded. Care should be taken to ensure integrity of the tissue.

Animals donating tissue are euthanized immediately prior to experiment initiation. The small intestine is excised from the animal and kept in ice cold Ringer's buffer pH 7.4 until mounted in diffusion chambers. As soon as possible after excision, the tissue is cut into an appropriately sized piece and placed over the diffusion chamber pins with the mucosal side down. The muscle layers are carefully stripped away using forceps. After the tissue is mounted the two half chambers are placed together and the donor and receiver sides filled with the appropriate pre-warmed (37°C) buffer. If NaviCyte chambers are used, the gas lift system is connected with 95% O_2/5% CO_2 flowing at ~5 – 15 ml/min (depending upon chamber volume) into each half chamber to maintain pH and mixing. Sampling begins 30 minutes after connection of the gas lift system.

The mean Pe and Standard Error of the Mean are determined for each study. Permeabilities from at least 6 chambers from 3 different animals are used in calculating the mean and Standard Error of the Mean.

In addition, the Pe of radiolabeled mannitol is determined simultaneously with the standard compound as a marker of intestinal integrity. Mannitol Pe values may be determined by concurrent diffusion using a donor buffer containing mannitol and the standard drug compound, or by continuing the experiment for 60 minutes after the last standard compound sample is collected using donor buffer containing mannitol and fresh receiver buffer containing no compounds.

Special experimental conditions are followed for certain standard compounds. This includes such conditions as a proton gradient, a sodium gradient, presence of glucose, etc. These conditions are listed in **Table 10** and are substituted or added to the general conditions listed above.

Table 10: Experimental Conditions

Standard Compound	Donor Concentration	Special Conditions
mannitol	1 mM	
D-glucose	10 mM	
hydrocortisone	0.01 µM	
etoposide	100 µM	drug dissolved in DMSO, DMSO concentration in buffer < 0.1%

Example 6: Physiologic-Based Mass Simulation Model

A. Design

A multi-compartment physiologic-based mass simulation model (the "mass model") was designed to integrate mass-flow relationships among GI compartments representing the stomach, duodenum, jejunum, ileum, and colon, and thus throughout the GI tract, and to characterize drug movement in units of mass into peripheral compartments. Converters that interrelated transfer rates and associated rate constants (k), which in turn were modified by various factors including pH, solubility profiles, compartment surface area and drug permeability were incorporated to account for drug movement among compartments. A plasma kinetics model also was included for validation purposes and for correlating clinical plasma data to the mass model. Converters also were used for unit conversion.

Gancyclovir was chosen to develop and test the mass model. Gancyclovir exhibits no *in vivo* biotransformation and is poorly absorbed. Thus, the mass model assumes no metabolism or protein binding. Additionally, dissolution rate and delivery system were not used in the mass model as modifying parameters of drug absorption, i.e., drug assumed to be completely dissolved in the stomach and solubilized according to its solubility profile.

Surface area values for each compartment of the mass model represented a "functional surface area," as opposed to an absolute value. A functional surface area was utilized since (1) fluids entering the gastrointestinal compartments do not cover the surfaces of the compartment instantaneously, but rather over a time course; and (2) solubilized drug within the fluid is not ideally presented to all absorptive areas. Functional surface areas for each compartment were calculated by solving **Equation** 6 for the area using various data inputs from the literature.

$$P \bullet A \bullet S_p = \partial M/\partial t \qquad \text{(Eq. 6)}$$

Where P is the permeability coefficient, A is the surface area of the membrane, S_p is the solubility of the drug in the relevant segment of the intestine, and $\partial M/\partial t$ is drug flux, where flux $\partial M/\partial t$ is determined from the permeability of the drug in the particular intestinal compartment, the surface area covered by drug solution and the solubility of at the pH of the intestinal compartment.

For example, several studies have been conducted comparing permeability of various compounds (Rubas *et al.*, *Pharmaceutical Research*, Vol. 10, No. 1 (1993)). Mannitol, which has similar physicochemical properties to Gancyclovir, also has similar permeability characteristics and a bioavailability of approximately 10% in humans when it is orally administered. For mannitol, permeability is well characterized. Thus, data obtained from the literature related to permeability in each compartment, pH-dependent solubility and mass concentration relationships was used to solve Equation (6) for area. Thus, it was this area, and not the theoretical total surface area of each compartment, that was used as the functional area of a compartment, which represented a good approximation of *in vivo* surface area relationships for initial model building.

Permeability values were obtained from published *in vitro* cell diffusion experiments and were accounted for by converters that modified luminal and peripheral flow (K12) for each compartment. For solubility, a solubility curve was used based on experimental data available in the literature. pH was then isolated in a separate converter to modify the solubility curve for the particular compartment. In contrast, for validation purposes, an absolute solubility value was used and pH was entered as 1 to isolate that converter from the validation model.

Absorption "transfer" rates among each two compartment sub-system were collected into a separate flow representing total absorption rate, which in turn was collected into a compartment representing the total amount of drug absorbed for each GI tract compartment, namely, stomach, duodenum, jejunum, ileum, and colon. Absorption rates among stomach, duodenum, jejunum, ileum, and colon modules were connected by flows modified by the associated rate constants between each GI segment.

For validation purposes, a plasma kinetics model was integrated with the mass-flow compartments by linking the total absorption rate to a flow representing the absorption rate constant, which in turn fed into the central plasma compartment. A standard two-compartment plasma kinetics model (Ramsay, *European Journal of Pharmaceutics and Biopharmaceutics,* Vol. 37, No. 3 (1991)) was used for this purpose. (See Figures 5 and 6) The plasma kinetics model incorporated first order transfers between the blood compartment and peripheral compartment. Two flows were used and set up as first order systems and thus different rate constants were applied in each direction. Compartment values were represented as mass units. Blood volume was input in a converter, which modified a converter for concentration along with the mass compartment. An elimination rate constant was also obtained form the literature in a first order process. In addition, while most drugs are given in milligram doses, plasma concentrations are reported in microgram or nanogram per milliliter. This is done since compounds are distributed rapidly into a large volume after entering the blood resulting in a concentration of drug in systemic circulation that is quite low with respect to the concentration at the site of administration. Accordingly, an additional converter was added to convert milligram units to nanogram or microgram units expected for concentrations of the test compound based on human bioavailability data. A compartment also was added to collect elimination data.

B. Mass Model Parameters

Parameters and associated values of the mass model include pH, solubility, permeability, and intestinal transit, and are illustrated in **Table 11**.

Table 11: Mass Model Parameters/Values

Parameter	Value
Dose	1000mg
dt	0.125
Run Time	24 hrs
ka assumed (mass transit)	2.8 or 3
Stomach	
Area	50 cm^2
Solubility	31 mg/ml
Permeability	1.1 X 10^{-6} cm/sec
Duodenum	
Area	125 cm^2
Solubility	3.65 mg/ml
Permeability	1.1 X 10^{-6} cm/sec
Jejunum	
Area	182 cm^2
Solubility	3.65 mg/ml
Permeability	2.17 x10^{-6} cm/sec
Ileum	
Area	102 cm^2
Solubility	3.65 mg/ml
Permeability	4.06x 10^{-6} cm/sec
Colon	
Area	138 cm^2
Solubility	3.65 mg/ml
Permeability	3.80 10^{-6} cm/sec
Plasma Kinetics	
k_{12}	0.839
k_{21}	0.670

k_{elim}	0.161
Fluid Volume	76,800 ml

The mass model also was tested by inputting values derived from the literature (Gibaldi *et al.*, *Pharmacokinetics*, pp. 284-288, Marcell Dekker (1975)) into the plasma kinetics model. These values are shown in **Table 12**.

Table 12: Values for Plasma Kinetic Module

Dose	1g
1505a	2.718 h^{-1}
1505b	0.254 h^{-1}
k_{21}	0.3737h^{-1}
k_{12}	0.7509h^{-1}
k_{10}	1.3474h^{-1}
V_p	20.1241

Example 7: Testing and Validation Mass Model

The mass model was tested using parameters shown in **Table 11** with an initial dose of 1000 mg over a time course of 24 hours. AUC, C_{max}, T_{max}, and $T_{1/2}$ were simulated using various doses (New Drug Application for Gancyclovir Sodium, Syntex (USA), (obtained from the FDA under the Freedom of Information Act (FIA)) and compared to human clinical data obtained for Gancyclovir. Bioavailability simulated by the mass model for Gancyclovir was approximately 6%. Compared to human clinical data, obtained for two Phase I clinical studies (designated here as ICM 1505 and 1505b), bioavailability of fasted patients in clinical trials typically ranged from 3-20%. The mass model also was tested using a plasma kinetics validation model illustrated in **Figure 8**.

Figure 16 shows the area under the concentration time curve for a 1000 mg dose of Gancyclovir, Tmax = 1.4 hrs, Cmax = .51 ng/ml., using the mass model, as compared to clinical study data of ICM 1505 and 1505b. The results demonstrate that the mass model underestimated plasma concentration during the post-absorptive period. **Table 13** shows comparison of some values between clinical studies and those predicted by the mass model. The clinical studies also used a 70Kg body weight for normalization of concentrations.

Table 13: Comparison of Mass Model to Clinical data

Parameter	Mass Model	Clinical 1505a	Clinical 1505b
Cmax (mcg/ml)	0.51	0.55	0.59
Tmax (hrs)	1.40	1.43	1.43

Example 8: Physiologic-Based Volume Simulation Model

A. Design

A physiologic-based simulation model for incorporating fluid volume flux and GI transit (the "volume model") was developed for integration with the mass model to account for changes in absorption resulting from fluid absorption/secretion and transit, and thus apparent drug concentration. The volume model was constructed so that fluid enters a compartment and was absorbed by a first order process based on an absorption rate for that fluid. Movement of fluid between compartments was dependent on a zero or first order fluid transit rate.

B. Volume Model Parameters

As a starting point for the volume model, values were obtained from literature that described in general terms absorption and secretion of fluid throughout the body (Change *et al.*, *Gastrointestinal, Hepatobiliary and Nutritional Physiology*, Chapter 5, p. 92, Lippincott-Raven (1996)). Values representing total intake of fluid per day and total secretion of fluid per day were modeled into the system normalized linearly to increments of dt for the model. To permit for changes in dt for the model, the values were entered as pulses. Values used in the volume model are shown in **Table 14**.

Table 14: Volume Model Parameters/Values

Source	ml/24hrs	ml/0.1hrs
Intake/Secretion		
Stomach	6500	27.08
Orally	2000	8.33
Salivary Glands	1500	6.25
Stomach	2500	10.42
Duodenum	2000	8.33
Bile	500	2.08
Pancreas	1500	6.25
Jejunum/Ileum	1000	4.17
Jejunum	641	2.67
Ileum	359	1.50
Colon	0	0
Total	9000	337.57.5
Absorption		
Duodenum	2598	10.82
Jejunum	3783	15.76
Ileum	2120	8.83
Colon	400	1.67
Total	8900	37.09
Note: Values for compartments based on %total intestinal area		

Where data was only available for a series of compartments, values were assigned to each compartment based on the percentage of the total area for that series (e.g. secretions for jejunum and ileum and absorption for parts of the small intestine). The model was set as two flows between the blood (serosal) side of the compartment and the compartment itself. Each flow represented the rate constant for secretion and fluid absorption.

For development purposes, absorption and stomach secretion were assumed to be zero order when using values from **Table 14** for both flows. Also, daily volume for fluid entry into the stomach was entered as a pulse according to the dt values shown in **Table 14**. Thus, total intake and secretions of fluid was modeled as a pulse occurring every 6 minutes throughout a 24 hour period. Initial volume in the stomach also was set up as a pulse of the total oral intake, salivary excretion, and stomach secretion over each dt increment.

Example 9: Testing and Validation of Volume Model

To test movement of fluid between compartments the volume model was modified to approximate zero order fluid transit or emptying and isolated from the mass component of the model. Initial values of 1000 ml and 250 ml were used for testing.

Example 10: Physiologic-Based Mass-Volume Simulation Model

A. Design

A physiologic-based simulation model integrating the mass and volume models (the "mass-volume model") was constructed to integrate complex mass and fluid flow relationships. The integrated mass-volume model also included compartments to characterize drug movement into peripheral compartments. A plasma kinetics model for training/validation purposes also was included. The basic design for the integrated mass-volume model, linked to the plasma kinetics model shown in **Figure 8**, is illustrated in **Figure 11.**

Volume for a compartment was added as a product to obtain the amount of drug solubilized at a time increment volume. Additionally, an "IF ... THEN ... ELSE" control statement was added to prevent the equation from indicating that more drug was solubilized than dosed. Thus, the integrated mass-volume model shows the mass of drug in the stomach connected to the absorption rate constant as well as the volume compartment.

Mass and fluid transit rate constants of 2.8 and 3 for the stomach were calculated from values obtained from the literature for Gancyclovir (Syntex, Clinical Studies ICM 1653 and 1774, FDA NDA available data and Bachrach *et al.*, Functional and Diagnostic Aspects of the Upper Digestive Tract, *Digestive System, Part I, Upper Digestive Tract*, Netter (1989)), and determined for each of the remaining compartments to approximate mass and fluid movement.

B. Mass-Volume Model Parameters

Parameters and associated values and equations were systematically varied or as described above for individual mass and volume models; an example of the equations and parameters employed in the mass-volume model are shown in **Appendix 1**. Dissolution rate and delivery system (controlled release device/formulation) were excluded from in the mass-volume model, and thus the model assumes a test compound is immediately in solution in the stomach.

Example 11: Testing and Validation of Mass-Volume Model

The mass-volume model was tested using the equations and parameters shown in **Appendix 1**. These parameters included the pulsed estimate of fluid absorption and gastrointestinal secretions, and rate constants extracted from the literature. Alternate sets of parameters for fluid absorption and secretions also were tested. For example, simple zero and first order rate constants of 1 or a sequential integer and various doses were evaluated for comparison to human clinical data.

Figure 17 shows the area under the concentration time curve for a 1000 mg dose of Gancyclovir, Tmax = 1.1875 hrs, Cmax = .54 mcg/ml., using the mass-volume model of **Figure 11** with the estimated absorption and secretion rates, relationships, and values of **Appendix 1**, as compared to clinical study data of ICM 1505 and 1505b. The data is now less favorable for Tmax but more favorable for AUC compared to the mass model. These results demonstrate that the mass model underestimated plasma concentration during the post-absorptive period, while the combined mass-volume model appeared to overestimate it.

The mass-volume model was modified to incorporate simple zero and first order absorption and secretion. This model was then run using an initial volume of 250 ml and also 4 administrations of 250 ml water as done during clinical studies. Results were similar to the results shown in **Figure 17**, but with slightly higher absorption.

The mass-volume model also was run using the following combinations of data input: (1) doses of 500 mg, 750 mg, 1000 mg at qid, bid, and tid dosing; (2) initial volumes of 250 ml, 500 ml, 1000 ml; (3) varying absorption and secretion rates based on differing assumptions for daily secretion and fluid intake; (4) varying pH values in the various compartments; and (5) simulation of food intake and fasting conditions. Correlation was very good with some clinical data and less than optimal with others. Correlation with theoretical estimations also varied from very good to poor.

Collectively, the mass-volume model represented an improvement over the individual mass and volume models in that it provided a better approximation of *in vivo* conditions. While the simpler mass-model correlated better with clinical data, the integrated mass-volume model was more sensitive to changes in the various input parameters, physiological conditions and underlying constants, and thus a more rigorous model of the GI tract.

Example 12: Physiologic-Based GI tract Simulation Model

A. Design

The mass-volume model was selectively improved in a stepwise fashion to create an integrated physiologic-based simulation model of the GI tract of a mammal (the "GI model") capable of compound-independent prediction of oral absorption with a high level of accuracy. The model was developed to be flexible. That is, it was designed so that additional physiological factors that influence oral absorption could be identified and incorporated into the model as needed to improve the quality of the prediction for a diverse set of test compounds. Additionally, the GI model was developed to minimize input data requirements.

The basic approach involved generation, testing and integration of a GI transit model (**Figure 20**), a pH-dependent solubility and dissolution model (**Figure 21**), and an absorption model (**Figure 22**), as well as underlying equations and parameters, constants, calculated parameters, and rules by which a given simulation is to proceed. A controlled release device and formulation compartment also was included. A graphical compartment-flow model of the integrated GI model is illustrated in **Figure 24** (without converters, ghost or connectors) and **Figure 25** (with converters, ghost and connectors). Parameter inputs, calculations and outputs are illustrated in **Figures 29-39**. An abbreviation key for the GI model is provided as **Appendix 3**.

The GI model also incorporated additional features to improve the predictive power and versatility of the simulation model. One feature was the development and incorporation of regression analysis derived adjustment parameters based on analysis and processing of human clinical data and *in vitro* data for a diverse set of compounds. The adjustment parameters were utilized as constants in the GI model, and thus modify underlying equations of the model. A second feature was development and incorporation of regional permeability correlation parameters and equations that permitted estimation of values for segments of the model that were missing user provided input values for corresponding parameters. This facilitates prediction of oral drug absorption when permeability values or other parameter for a given compound are provided for a limited number of GI segments, for example, when cell-based input data, such permeability data derived from Caco-2 cells is used to provide permeability input data of colon. Another feature was development and incorporation of parameters and calculations to account for transport mechanism and thus transport-specific variations in compound absorption. Another feature was incorporation of the ability to isolate and evaluate specific regional absorption events related to dissolution and mass transit. Also, the GI model was developed to separate absorption into the portal vein (FDp) from hepatic metabolism, so as to account for individual primary barriers to absorption.

B. GI Model Equations, Rules and Parameters

1. General Equations For GI Model:

Various differential equations and rules utilized for the GI tract model are provided below. For the equations, adjustment parameters are designated by the letter Z.

Transit time:

First order transit process

$$\frac{dA}{dt} = k_{TT}[A] \qquad \text{(Eq. 7)}$$

dA/dt = rate of transit (or absorption), k_{TT} = rate of constant, A = amount (compound or water) in proximal compartment.

Rate constant calculation

$$k_{TT} = \frac{\ln 10}{TT_{ADJ}} \qquad \text{(Eq. 8)}$$

TT_{ADJ} = adjusted transit time

$$TT_{ADJ} = (TT_p \cdot Z_{TT} \cdot User_{TT}) \qquad \text{(Eq. 9)}$$

TT_p = physiological transit time, Z_{TT} = transit time adjustment parameter, $User_{TT}$ = User controlled adjustment to transit time.

K_{TT} is a regionally dependent parameter, i.e. different rate constants are used for each region of the GI tract.

Fluid volume absorption/resorption:

$$\frac{dA}{dt} = k_{VA}[A] \qquad \text{(Eq. 10)}$$

dA/dt = rate of absortpion, k_{VA} = rate constant, A = amount of fluid (water) in the compartment

$$k_{VAZ} = k_{emp} \cdot Z_{VA} \qquad \text{(Eq. 11)}$$

Z_{VA} = volume absorption adjustment parameter, k_{emp} is determined emperically to match human fluid absorption *in vivo*.

Dissolution and Solubility:

Dissolution rate (regionally dependent)

$$\frac{d(A)}{dt} = k_D \cdot Z_D \cdot Mass \cdot (S_{ADJ} - C) \qquad \text{(Eq. 12)}$$

A = Amount dissolved, k_D = User supplied dissolution rate constant, Z_D = Dissolution rate adjustment parameter, S_{ADJ} = solubility, C = concentration

Solubility (regionally dependent)

$$S_{ADJ} = \frac{(s_N - s_{n-1})}{(pH_n - pH_{n-1})}(pH - pH_{n-1}) + S_{n-1} \qquad \text{(Eq. 13)}$$

S_{ADJ} = Solubility, S_n = user supplied solubility $\{S_1 \ldots S_5\}$, pH_n = user supplied pH values $\{pH_1 \ldots pH_5\}$ corresponding to user supplied solubilities, pH = pH value appropriate to region of the system, such as GI tract. n is selected such that $pH_n > pH$, and $pH_{n-1} < pH$. If any of $pH_1 \ldots pH_5$ are equal to pH, the corresponding S_n is used as the solubility.

Concentration (regionally dependent)

$$C = \frac{S_{ADJ}}{V} \qquad \text{(Eq. 14)}$$

C = concentration of soluble drug, V = volume of fluid

Flux/Absorption:

$$J = P_{ADJ} \,.\, SA_{ADJ} \,.\, C \qquad \text{(Eq. 15)}$$

J = flux, P_{ADJ} = Adjusted permeability, SA_{ADJ} = Adjusted surface area available for absorption, C = concentration

$$P_{ADJ} = \left(\frac{2}{1 + Z_{EFF}}\right) \cdot P_m \cdot Z_F \cdot 3600 + \frac{Z_{ACT} \cdot P_c \cdot 3600}{1 + \dfrac{C}{K_m}} \qquad \text{(Eq. 16)}$$

Z_{EFF} = Efflux transport adjustment parameter, P_m = passive membrane permeability, Z_F = passive permeability or flux adjustment parameter, Z_{ACT} = active permeability adjustment parameter, P_c = active carrier permeability, C = concentration, K_m = Michaelis-Menten kinetic parameter.

Regional Permeability Correlation

Any regional permeability, P_m, can be calculated using any number of other provided permeabilities.

$$\ln P_a = C + A \cdot \ln \frac{1}{P_b} + B \cdot \ln\left(\frac{1}{P_b}\right)^2 \qquad \text{(Eq. 17)}$$

P_a = permeability calculated using the regional correlation, P_b = permeability provided by the user, and A, B, and C = correlation coefficients fitted to determine correlation.

By way of example, rules utilized for a GI tract model of the PK tool and method of the invention include the following general processes.

2. General Processes For Rule Generation:

1. GI transit. The transit of drug compound and fluid volume are somewhat controlled and the transit of formulations and/or controlled release devices is much more strictly controlled.

2. Controlled Drug Release. The release of drug from the dosage form must be controlled such that drug is released into the correct intestinal region at the appropriate time.

3. Dissolution. A comparison between the concentration and the solubility must be made to determine if additional insoluble compound will dissolve, or if compound already dissolved must precipitate to insoluble drug due to solubility limitations.

4. Absorption. Mathematically, absorption may occur when physiologically it is impossible, e.g. when the volume in the colon becomes low enough that any dissolved drug will be within fluid contained in other solid waste also present in the colon and therefore unavailable for absorption. IF...THEN production rules control these situations.

5. Permeability calculations. To estimate unprovided permeability values from provided permeability values logical evaluations must be made to determine the correct equations necessary to make the correlations.

6. Concentration calculations. The concentration in the intestine cannot exceed the solubility for that particular region. If it does, an incorrect flux will be calculated. IF...THEN production rules are used to ensure the correct concentration is used in the flux calculation.

7. Mathematical anomalies. At certain times during the simulation (especially early and late in the simulation) some compartments, flow regulators, or converters used in other calculations may have a value of 0 which will result in a computational error, e.g. division by 0. Production of rules are used to identify these situations and avoid the errors.

The following table lists the specific processes, conditions, results that control statement rules, e.g., IF...THEN production rules, are used to control. Generally, separate rules used for each region of the GI tract and are combined into one line in the table.

Table 15: Rules for Physiologic-Based GI tract Simulation Model

Process	Condition	Result in True	Result if False	Comments
GI Transit of drug compound or fluid volume	Time < 4 hours	No transit to waste	Transit to waste by first order process	Applies to GI regions using different values for the condition.
GI Transit of formulations or controlled release devices	Time, cumulative physiol. transit time	no transit to next compartment	Immediate transit to next compartment	The rate constant for first order transit is set exceedingly large to provide near instantaneous transit.
Controlled release	Time to reach GI region < Time < Time to exit GI region	Drug is released from dosage form to GI region	No drug release into that GI region	Drug is released according to user provided release profile.
Dissolution	Soluble drug/volume (concentration) < Solubility	Drug moves from insoluble to soluble compartment according to dissolution rate	Drug moves from soluble to insoluble compartment according to precipitation rate	Precipitation rate is set to provide near instantaneous precipitation without causing "overshoot".
Absorption	Volume < 1 x 10^{-6} ml AND Mass < 1 x 10^{-8} mg	No absorption, i.e. concentration = 0	Absorption by flux equation	
Permeability Calculations	Duodenum, Jejunum, and Ileum Permeabilities all provided	Use provided Permeabilities	Estimate unprovided permeabilities from provided permeabilities	1 or 2 permeabilities can be used to calculate unprovided permeabilities
Concentration Calculation	Concentration < Solubility	Concentration used in flux equation	Solubility used in flux equation	
Mathematical anomalies	Volume = 0	Dissolution rate = 0	Dissolution rate calculated by Noyes-Whitney equation	Dissolution given as an example. Similar condications are provided for concentration calculations and other processes.

Exemplary equations, rules, parameters and initial values for the graphical compartment-flow model and various sub-models of the integrated GI model illustrated in **Figures 20-25** and **29-39** are provided in **Appendix 4**, as related to the abbreviation key provided as **Appendix 3**. Various aspects of the physiological, adjustment and regional correlation parameters employed in the GI model and their development are described in further detail below.

1. Physiological Parameters

Physiological parameters of the GI model included physiological ranges reported in the literature **(Table 17)** as well as specific values utilized in the model and compiled for each of five regions of the gastrointestinal tract (stomach, duodenum, jejunum, ileum and colon)**(Table 16)**. These included values related to pH, transit time, surface area, and volume parameters.

Table 16: Physiological Parameters Employed In GI Model

	pH[a]	Initial Volumes (ml)	Surface Area (cm^2) [b]	Average Transit time (hr) [c]	Volume Transfer Rates (t_{90}) (hr^{-1})[c]	New Water Absorption Rates* (hr^1) [d]
Stomach	1.5	100	NA	0.5	4.6	0
Duodenum	6.0	0	150	0.225	10.8	0
Jejunum	6.5	0	1000	1.5	1.54	1.75
Ileum	7.0	0	1000	1.5	1.54	1.75
Colon	6.5	0	850	24	0.094	0.1

*Water absorption rate parameters were set so that cumulative water absorption from each region using the GI model were in agreement with values listed in **Table 17**.

Table 17: Physiological Parameters Employed In GI Model

	pH^a	Initial Volumes (ml)	Surface Area (cm^2) ^{b}	Average Transit time (hr) ^{c}	Volume Transfer Rates (t_{90}) (hr^{-1})^c	New Water Absorption Rates (hr^1) ^{d}
Stomach	1.0-2.5	100	NA	0.5-3.0	0.8-4.6	0
Duodenum	4.0-6.4	0	147-168	0.20-0.25	9.2-11.5	0
Jejunum	4.4-6.4	0	913.5-1044	1.0-2.0	1.15-2.3	4.0-4.5
Ileum	6.8-7.4	0	913.5-1044	1.2-1.5	1.54-1.9	2.4-2.7
Colon	5.5-7.0	0	763-872	18-36	0.064-0.13	1.4-1.6

a) Lui et al. *J Pharm Sci* 1986;75(3):271-4; Youngberg et al. *Dig Dis Sci* 1987;32(5):472-80; Charman et al. *J Pharm Sci* 1997;86(3):269-82; Langguth et al. *Biopharm Drug Dispos* 1994;15(9):719-46; Kararli TT. *Biopharm Drug Dispos* 1995;16(5):351-80;

b) Wagner JG. *J Pharm Sci* 1961;50(5):59-87; Ho NF, Park JY, Ni PF, et al. Crouthamel W, Sarapu AC, editors. *Animal Models For Oral Drug Delivery In Man: In Situ And In vivo Approaches.* Washington, D.C. American Pharmaceutical Association, 1983; 2, Advancing quantitative and mechanistic approaches in interfacing gastrointestinal drug absorption studies in animals and humans. p. 27-106;

c) Ho et al. Crouthamel W, Sarapu AC, editors. *Animal Models For Oral Drug Delivery In Man: In Situ And In vivo Approaches.* Washington, D.C. American Pharmaceutical Association, 1983; 2, Advancing quantitative and mechanistic approaches in interfacing gastrointestinal drug absorption studies in animals and humans. p. 27-106; Oberle et al. *Journal of Pharmacokinetics & Biopharmaceutics* 1987;15:529-44; Davis SS. *S T P Pharma* 1986;22:1015-22; Davis et al. *Gut* 1986;27:886-92;

d) Turnberg LA. *Digestion* (1973) 9:357-81.

2. Adjustment Parameters

Differences between *in vitro* and *in vivo* conditions, as well as differences between *in vivo* conditions for one species of mammal and a second hamper accurate prediction of absorption using a simulation approach. For example, *in vitro* dissolution rate may or may not be comparable to dissolution rates existing *in vivo*, or, the permeability in rabbits may or may not be comparable to the permeability in humans.

To compensate for such differences, a set of selectively optimized adjustment parameters were developed. These parameters were designed to be utilized as constants that modify the underlying equations of specific compartments of the GI model to permit automatic correlation of input data to output data as well as facilitate accurate prediction of oral absorption for a diverse set of compounds. For example, the differential equation utilized to calculate fluid volume absorption/resorption employs a rate constant obtained from an equation that is modified by a volume absorption adjustment parameter Z_{VA} (see Eq. 11) Listed below **(Table 18)** are examples of parameters that can be used to adjust parameters and equations as well as those which can be added or removed to a given model if necessary.

Table 18: Adjustment Parameters

Compartment	Segment
Regional fluid absorption	stomach duodenum jejunum ileum colon
Flux/Permeability	duodenum jejunum ileum colon
Active/Carrier mediated Transport (absorption)	duodenum jejunum ileum colon
Compound Efflux (secretion)	duodenum jejunum ileum colon
Transfer rates	stomach to duodenum duodenum to jejunum jejunum to ileum ileum to colon colon to waste
Surface Area	duodenum jejunum ileum colon

The adjustment parameters were developed and optimized using a stepwise selective optimization process. Initial adjustment parameters were developed for correlation between humans and rabbit as follows. Two primary sets of data were used: 1) FDp and best fit plasma profiles from *in vivo* clinical pharmacokinetic (PK) data, and 2) simulated FDp and plasma profiles generated from the GI model. The FDp and best fit plasma profiles from *in vivo* PK data was obtained by analyzing and processing IV and PO data from humans for the test set of compounds described in **Example 2** using a regression-based curve fitting algorithm to determine the best fit curve that matched the actual clinical plasma profiles. The second set of data was generated using a developmental GI model.

In vitro data (permeability, solubility, dissolution rate, and dose) were used as inputs into the GI model with the adjustment parameters set to some initial value previously determined to provide reasonably predictable values for FDp. The GI model was used to provide FDp data for each test compound. The FDp data generated from the GI model also was used as input data into an IV/PO PK model, such as the one shown in **Figure 18**, to determine plasma profiles.

The PO input to the IV/PO PK model of **Figure 18** used for fitting clinical data is an error function and shown in **Equation 18**.

$$F = \frac{D \cdot FDp}{2} \left| 1 - erf \frac{\frac{1 - \frac{t}{t_{50}}}{2}}{\frac{1}{P_e} \cdot \sqrt{\frac{t}{t_{50}}}} \right|$$

(Eq. 18)

Where D is the dose of drug delivered to the intestine, t is time in minutes, t50 is the time for 50% of the drug to be absorbed, and Pe is a parameter (Peclet number) related to the slope of the linear portion of the absorption curve.

When fitting the data, all available *in vivo* PK data (multiple intravenous (IV) dosing and multiple oral (PO) dosing) was analyzed simultaneously using the IV/PO PK model of **Figure 18**. The data were weighted by 1/Standard Error of the Mean (SEM) or by 1/Concentration^2.

The initial adjustment parameter values were determined empirically. Using a limited set of compounds and corresponding *in vitro* data from rabbit tissue, the adjustment parameters were manually varied to obtain FDp values that were reasonably consistent with the actual PK data. After the initial values were determined, the GI model developed using STELLA® was converted to a program file readable by a program having fitting algorithm, such as KINETICA™. The initial adjustment parameters were then simultaneously fit using non-linear regression analysis in a stepwise manner to determine optimized values (i.e., best fit values) for the adjustment parameters. Within each step, a few parameters were selected for optimization by simultaneous fitting. The fitting was approached using an iterative process, where selected adjustment parameters were varied systematically such that the deviation of the GI model determined absorption from the actual PK determined absorption was minimized. Once the deviation was reduced to a satisfactory level, few more parameters were then selected and optimized. The process was continued until all parameters were successfully optimized. The new parameters were then placed into the GI model and the FDp determined for each compound which is compared to the PK FDp to establish the goodness of fit. This process was repeated until an acceptable goodness of fit was established. Using this approach, adjustment parameters were developed to correlate, for example, *in vitro* solubility, dissolution, dose and permeability in rabbits to *in vivo* human absorption. Although FDp was employed as the reference for deviation, the actual measurement of absorption can be evaluated using any number of parameters, such as plasma levels, absorption constants, or others. Moreover, it will be appreciated that many sets of adjustment parameters may be developed and established. For instance, other sets of adjustment parameters may be established to correlate dog, rat, monkey or other species permeability data to human, dog, rat, rabbit, monkey, or other animal *in vivo* absorption.

3. Regional Permeability Correlation Parameters

Since Pe in all intestinal regions may not be available, for instance when cell monolayer data is employed to determine Pe in colon, a correlation was developed that provides a reasonable prediction of unknown Pe values in the other intestinal regions.

An objective was to establish a correlation between regional permeabilities that allowed prediction of permeability in the duodenum, jejunum or ileum using known permeabilities in one or two of the other regions.

Correlation development involved obtention of regional permeability values in intestinal tissue from the literature and experimentally using methods consistent with the experimental protocols as described in **Examples 4-5**.

The regional correlation parameters were estimated using a polynomial equation developed for this purpose (Equation 17). Any regional permeability, P_m, can be calculated using any number of other provided permeabilities.

The regional correlation parameter function was incorporated into the GI model using a logic function module. A control statement was utilized to regulate activation of the regional correlation parameter estimation function when a user provides less than the total number of permeability values for the segments of the GI tract.

The following **(Table 19)** shows correlations that were established along with the corresponding correlation coefficient. Correlations were accomplished by data transformation and fitting to a non-linear function.

Table 19: Results of Regional Correlation

Variable		Correlation Coefficient
Dependent	Independent	
Duodenum	Jejunum	0.870
Duodenum	Ileum	0.906
Jejunum	Duodenum	0.858
Jejunum	Ileum	0.914
Ileum	Duodenum	0.855
Ileum	Jejunum	0.894

As an example of the capability of the correlation, two of the above correlations were evaluated by estimating the permeability in the duodenum and jejunum using ileum Pe values. The compounds chosen were those for which complete Pe values were available.

The error and % error for the permeability calculations were determined by comparing predicted values to the known permeabilities **(Table 20)**.

Table 20: Evaluation of Regional Correlations

Compound	Intestinal Region			
	Duodenum		Jejunum	
	Error	%Error	Error	%Error
Compound α1	-4.64E-07	-46.36	2.42E-07	35.03
Compound α2	6.37E-08	5.79	-1.11E-07	-5.14
Compound α3	3.10E-07	114.91	-8.38E-07	-45.28
Compound α4	1.18E-05	196.00	-5.40E-06	-16.38

The above results demonstrate that the regional correlation parameter function of the GI model was able to accurately predict Pe values for compounds within the initial data set (i.e., high r^2).

Example 13: Validation and Testing of GI Model

To demonstrate that the physiological parameters of the model were operating in a logical manner consistent with expected behavior *in vivo*, the parameters were varied and the effect on output monitored. For example, a decrease in the surface area available for absorption should result in a decrease in the amount of compound absorbed. Thus, the physiological parameters of the model were varied by increasing and/or decreasing their values. The effect of these variations on the rate, as measured by T50 (time for 50% absorption), and extent, as measured by FDp, were simulated. **Table 21** shows the physiological parameters that were varied and the expected effect on FDp and T50.

Table 21: Physiological Parameter Variations*

Parameter	Range evaluated	Expected effect	
Surface Area or Permeability	0.05 to 10 X Normal* $1\text{x}10^{-7}$ to $1\text{s}10^{-5}$ cm/s	Increase in: Surface Area or Permeability	Increase FDp Decrease T50
GI Transit Time	0.05 to 10 X Normal*	Increase in: GI Transit Time	Increase FDp Increase T50
Dissolution Rate	0.05 to 10 X Normal*	Increase in: Dissolution Rate	Increase FDp Decreased T50
Solubility	1 to 100 mg/ml	Increase in: Solubility	Increase FDp Decrease T50

*Normal values used in the model are listed in **Example 12**. In each case, only the parameter chosen was varied, all other parameters were held constant.

All effects on FDp and T50 were as expected with the changes in the physiological parameters. While not all of the ranges were in the physiological range, the lower part of the range was included to assure that the model would limit to zero FDp as the various parameters approached zero.

The basic structure of the GI model also was assessed by comparing its ability to predict, from dose and *in vitro* solubility and rabbit tissue permeability data, the rate and extent of oral drug absorption in humans and dogs for several drugs, including atenolol, ganciclovir, verapamil, and naproxin. These compounds were chosen for their well known and diverse *in vivo* absorption properties and interspecies absorption variability. By changing the physiological parameter values of the simulation model so that they corresponded to the species under investigation, but not changing the model structure, i.e., compartment, flow regulator, converter relationships, efficacy of the model structure could be evaluated. Initial parameter values for dog and human were derived from the literature. Adjustment parameters were used to build the correlation between the *in vitro* data and *in vivo* absorption. For all four drugs, the GI model accurately predicted FDp for both dog and human.

To assess the basic power of the GI model for predicting oral drug absorption, the model was tested by simulating FDp as a function of time so as to separate absorption across intestinal tissue from first pass metabolism and drug concentration in systemic circulation. Accordingly, methods were developed and used to determine

FDp from clinical plasma data so that transport across the intestinal tissue could be determined. This was accomplished by simultaneously fitting clinical pharmacokinetic data (PO and IV) to the two compartment open IV/PO PK model illustrated as a compartment-flow model in **Figure 18**. Elimination was from the central compartment. Input from oral doses was into a pre-systemic compartment (for metabolism) which was in equilibrium with the central compartment. FDp was determined simultaneously for each oral dose. Clinical pharmacokinetic data fitted to the IV/PO PK model demonstrated the ability of the model to accurately determine blood levels in the central compartment.

The fitted clinical FDp data for a test set of compounds was then compared to FDp predicted by the GI model using both experimental *in vitro* values for permeability as input as well as estimated permeability values calculated by the model using the regional permeability correlation function. The permeability source of the test compounds are shown in **Table 22** below.

Table 22: Permeability Source of Test Compounds

Compound	Permeability source*
∝1	experimental
∝2	experimental
∝3	experimental
∝4	experimental
∝5	estimation
∝6	experimental
∝10	estimation
β1	estimation
β2	estimation
β3	estimation
β5	estimation
β6	estimation

*Experimental – permeability values for all intestinal segments were submitted. Estimation – permeability values were calculated using regional permeability correlation parameters.

Figures 48-52 are illustrative of the results of these tests. The physiological model was found to accurately predict FDp for the test set of compounds. The accuracy of the prediction is based on both rate and extent of absorption. Correlation of FDp extent between the clinical data and as predicted by the model for the test set of compounds yielded a collective regression coefficient (r^2) of greater than 0.92.

Example 14: Smoothing Functions for GI Model

In the *in vivo* physiological situation, permeability and pH do not change at distinct points or places within the GI tract (with the exception of the gastro-duodenal junction). For example, permeability of a drug in the duodenum may be measured at 1.5×10^{-6} cm/s and 2.5×10^{-6} cm/s in the jejunum, but there is no distinct point in the intestine where such an abrupt change exist. Since the GI model simulates five regions or segments of the GI tract, and each segment utilizes its own set of initial permeability and pH values, an abrupt change, as opposed to an incremental transition, is simulated for a dosage form or dissolved drug as it passes distally through the segmented GI tract.

To account for this phenomenon, and to generate a GI model that is as physiologically accurate as possible, smoothing functions were incorporated into the model. Pairs of exponential functions were used to adjust the permeability and pH values in each segment of the intestine. The functions were developed to be time/position dependent using the mean cumulative transit time as cues for adjustment. For example, prior to the cumulative transit time to reach the ileum ($C_{TT}I$), the ileum permeability will be equal to the user provided or regional correlation estimated jejunum permeability. As time approaches $C_{TT}I$, the ileum permeability will correspond to the exact average of the jejunum and ileum permeability at that point. Immediately after $C_{TT}I$, the ileum permeability continues to gradually decrease/increase exponentially until it reaches the user provided, or estimated, ileum permeability.

Two exponential functions were used in combination to effectively smooth the permeability and pH values. The GI model was adapted to employ Equation 19 as the time approaches a mean cumulative transit time (C_{TT}), and Equation 20 immediately after C_{TT}.

$$P = A - ke^{(\alpha t)} \qquad \text{(Eq. 19)}$$

$$P = B + ke^{-\alpha(t - TT)} \qquad \text{(Eq. 20)}$$

Where A = permeability or pH in the previous intestinal region or segment, B = permeability or pH in the latter region, k is defined in Equation 21, α = a constant used to determine the steepness of the transition between regions and is inversely proportional to the transit time of the region, t = time, and TT = cumulative transit time to exit the previous region.

$$k = 0.5(A - B)/e^{\alpha TT} \qquad \text{(Eq. 21)}$$

These smoothing functions were utilized to adjust permeability and pH at junctions of the stomach/duodenum, duodenum/jejunum, jejunum/ileum, and ileum/colon.

APPENDICIES

Appendix 1: Abbreviation Key for Mass-Volume Model

Abbreviation
Kf sd = associated rate constant for stomach and duodenum
Ka dj = associated rate constant for duodenum and jejunum
Ka ji = associated rate constant for jejunum and ileum
Ka ie = associated rate constant for ileum and colon
Ka co = associated rate constant for colon and excretion
SD trans = transfer rate between stomach and duodenum
DJ trans = transfer rate between duodenum and jejunum
JL trans = transfer rate between jejunum and ileum
IC trans = transfer rate between ileum and colon
Waste = transfer rate between colon and excretion
pH s = pH stomach
pH s2 = pH duodenum
pH s3 = pH jejunum
pH s4 = pH ileum
pH s5 = pH colon
sol profile = solubility profile for stomach
sol profile 2 = solubility profile for duodenum
sol profile 3 = solubility profile for jejunum

sol profile 4 = solubility profile for ileum

sol profile 5 = solubility profile for colon

stom ka = associated rate constant for stomach compartments 1 and 2

duo ka = associated rate constant for duodenum compartments 1 and 2

Jej ka = associated rate constant for jejunum compartments 1 and 2

Il ka = associated rate constant for ileum compartments 1 and 2

Colon ka = associated rate constant for colon compartments 1 and 2

SA stom = surface area of stomach

SA duo = surface area of duodenum

SA jej = surface area of jejunum

SA il = surface area of ileum

SA colon = surface area of colon

Perm stom = permeability of stomach

Perm duo = permeability of duodenum

Perm jej = permeability of jejunum

Perm il = permeability of ileum

Perm colon = permeability of colon

Ka sd = associated rate construct for stomach fluid absorption

Ka du = associated rate construct for duodeunm fluid absorption

Ka je = associated rate construct for jejunm fluid absorption

Ka il = associated rate construct for ileunm fluid absorption

Ka co = associated rate construct for colon fluid absorption
Note: other abbreviations adhere to above descriptors and are self explanatory

Appendix 2: Equations, Parameters and Values For Mass-Volume Model

amt_plasma(t) = amt_plasma(t - dt) + (trans_21 + ka - elimination - trans_12) * dt
INIT amt_plasma = 0

INFLOWS:
trans_21 = k21*comp_2
ka = tot_abs_rate

OUTFLOWS:
elimination = amt_plasma*k_elim
trans_12 = k12*amt_plasma
blood_side_col(t) = blood_side_col(t - dt) + (colon_ka_5) * dt
INIT blood_side_col = 0

INFLOWS:
colon_ka_5 = IF Vol_colon*sol_profile_5 >=Colon THEN Colon*SA_colon*perm_colon*3600 ELSE Vol_colon*sol_profile_5*SA_colon*perm_colon*3600
blood_side_dou(t) = blood_side_dou(t - dt) + (duo_ka) * dt
INIT blood_side_dou = 0

INFLOWS:
duo_ka = IF Vol_duod*sol_profile_2 >= duodenum THEN duodenum*SA_duo*perm_duo*3600 ELSE Vol_duod*sol_profile_2*SA_duo*perm_duo*3600
blood_side_il(t) = blood_side_il(t - dt) + (Il_ka) * dt
INIT blood_side_il = 0

INFLOWS:
Il_ka = IF Vol_ileum*sol_profile_4 >=Ileum THEN Ileum*SA_Il*perm_Il*3600 ELSE Vol_ileum*sol_profile_4*SA_Il*perm_Il*3600
blood_side_jej(t) = blood_side_jej(t - dt) + (Jej_ka) * dt
INIT blood_side_jej = 0

INFLOWS:
Jej_ka = IF Vol_jej*sol_profile_3 >=Jejunum THEN Jejunum*SA_jej*perm_jej *3600 ELSE Vol_jej*sol_profile_3*SA_jej*perm_jej*3600
blood_side_sto(t) = blood_side_sto(t - dt) + (stom_ka) * dt
INIT blood_side_sto = 0

INFLOWS:
stom_ka = IF Vol_stom*sol_profile >= Stomach THEN Stomach*SA_stom*perm_stom*3600 ELSE Vol_stom*sol_profile*SA_stom*perm_stom*3600
Colon(t) = Colon(t - dt) + (IC_trans - Waste - colon_ka_5) * dt
INIT Colon = 0

INFLOWS:

```
IC_trans = ka_ic*Ileum

OUTFLOWS:
Waste = ka_col*Colon
colon_ka_5 = IF Vol_colon*sol_profile_5 >=Colon THEN
Colon*SA_colon*perm_colon*3600 ELSE
Vol_colon*sol_profile_5*SA_colon*perm_colon*3600
comp_2(t) = comp_2(t - dt) + (trans_12 - trans_21) * dt
INIT comp_2 = 0

INFLOWS:
trans_12 = k12*amt_plasma

OUTFLOWS:
trans_21 = k21*comp_2
duodenum(t) = duodenum(t - dt) + (SD_trans - duo_ka - DJ_trans) * dt
INIT duodenum = 0

INFLOWS:
SD_trans = if Stomach >0 then kf_sd*Stomach else 0

OUTFLOWS:
duo_ka = IF Vol_duod*sol_profile_2 >= duodenum THEN
duodenum*SA_duo*perm_duo*3600 ELSE
Vol_duod*sol_profile_2*SA_duo*perm_duo*3600
DJ_trans = ka_dj*duodenum
excretion(t) = excretion(t - dt) + (vol_cw) * dt
INIT excretion = 0

INFLOWS:
vol_cw = Vol_colon*ka_col
excretion_2(t) = excretion_2(t - dt) + (Waste) * dt
INIT excretion_2 = 0

INFLOWS:
Waste = ka_col*Colon
Ileum(t) = Ileum(t - dt) + (JL_trans - IC_trans - Il_ka) * dt
INIT Ileum = 0

INFLOWS:
JL_trans = ka_ji*Jejunum

OUTFLOWS:
IC_trans = ka_ic*Ileum
Il_ka = IF Vol_ileum*sol_profile_4 >=Ileum THEN Ileum*SA_Il*perm_Il*3600
ELSE Vol_ileum*sol_profile_4*SA_Il*perm_Il*3600
Jejunum(t) = Jejunum(t - dt) + (DJ_trans - JL_trans - Jej_ka) * dt
INIT Jejunum = 0

INFLOWS:
```

```
DJ_trans = ka_dj*duodenum

OUTFLOWS:
JL_trans = ka_ji*Jejunum
Jej_ka = IF Vol_jej*sol_profile_3 >=Jejunum THEN Jejunum*SA_jej*perm_jej
*3600 ELSE Vol_jej*sol_profile_3*SA_jej*perm_jej*3600
serosal_col(t) = serosal_col(t - dt) + (Adsorp_col - col_secretion) * dt
INIT serosal_col = 0

INFLOWS:
Adsorp_col = PULSE(1.67,0,.1)+0*Vol_colon*ka_co

OUTFLOWS:
col_secretion = 0
serosal_dou(t) = serosal_dou(t - dt) + (Adsorp_Duo - duo_secretion) * dt
INIT serosal_dou = 0

INFLOWS:
Adsorp_Duo = PULSE(10.82,0,.1)+0*Vol_duod*ka_du

OUTFLOWS:
duo_secretion = PULSE(10.82,0,.1)
serosal_ill(t) = serosal_ill(t - dt) + (Adsorpt_ill - ile_secretion) * dt
INIT serosal_ill = 0

INFLOWS:
Adsorpt_ill = PULSE(8.83,0,.10)+0*Vol_ileum*ka_il

OUTFLOWS:
ile_secretion = PULSE(1.50,0,.1)
serosal_jej(t) = serosal_jej(t - dt) + (Adsorp_jej - jej_secretion) * dt
INIT serosal_jej = 0

INFLOWS:
Adsorp_jej = PULSE(15.76,0,.1)+0*Vol_jej*ka_je

OUTFLOWS:
jej_secretion = PULSE(2.67,0,.1)
serosal_sto(t) = serosal_sto(t - dt) + (Adsorp_Stom - Stom_Secretion) * dt
INIT serosal_sto = 0

INFLOWS:
Adsorp_Stom = 0*Vol_stom*ka_sd

OUTFLOWS:
Stom_Secretion = PULSE(16.67,0,.1)
Stomach(t) = Stomach(t - dt) + (- SD_trans - stom_ka) * dt
INIT Stomach = 1000

OUTFLOWS:
```

```
SD_trans = if Stomach >0 then kf_sd*Stomach else 0
stom_ka = IF Vol_stom*sol_profile >= Stomach THEN
Stomach*SA_stom*perm_stom*3600 ELSE
Vol_stom*sol_profile*SA_stom*perm_stom*3600
total_drug_absorbed(t) = total_drug_absorbed(t - dt) + (tot_abs_rate) * dt
INIT total_drug_absorbed = 0

INFLOWS:
tot_abs_rate = stom_ka+duo_ka+Jej_ka+Il_ka+colon_ka_5
Total_Elimination(t) = Total_Elimination(t - dt) + (elimination) * dt
INIT Total_Elimination = 0

INFLOWS:
elimination = amt_plasma*k_elim
Vol_colon(t) = Vol_colon(t - dt) + (vol_ij + col_secretion - vol_cw - Adsorp_col) * dt
INIT Vol_colon = 0

INFLOWS:
vol_ij = Vol_ileum*ka_ic
col_secretion = 0

OUTFLOWS:
vol_cw = Vol_colon*ka_col
Adsorp_col = PULSE(1.67,0,.1)+0*Vol_colon*ka_co
Vol_duod(t) = Vol_duod(t - dt) + (vol_sd + duo_secretion - voil_dj - Adsorp_Duo) *
dt
INIT Vol_duod = 0

INFLOWS:
vol_sd = kf_sd*Vol_stom
duo_secretion = PULSE(10.82,0,.1)

OUTFLOWS:
voil_dj = Vol_duod*ka_dj
Adsorp_Duo = PULSE(10.82,0,.1)+0*Vol_duod*ka_du
Vol_ileum(t) = Vol_ileum(t - dt) + (vol_ji + ile_secretion - Adsorpt_ill - vol_ij) * dt
INIT Vol_ileum = 0

INFLOWS:
vol_ji = Vol_jej*ka_ji
ile_secretion = PULSE(1.50,0,.1)

OUTFLOWS:
Adsorpt_ill = PULSE(8.83,0,.10)+0*Vol_ileum*ka_il
vol_ij = Vol_ileum*ka_ic
Vol_jej(t) = Vol_jej(t - dt) + (voil_dj + jej_secretion - vol_ji - Adsorp_jej) * dt
INIT Vol_jej = 0

INFLOWS:
voil_dj = Vol_duod*ka_dj
```

```
jej_secretion = PULSE(2.67,0,.1)

OUTFLOWS:
vol_ji = Vol_jej*ka_ji
Adsorp_jej = PULSE(15.76,0,.1)+0*Vol_jej*ka_je
Vol_stom(t) = Vol_stom(t - dt) + (Stom_Secretion - vol_sd - Adsorp_Stom) * dt
INIT Vol_stom = PULSE(8.33,0,.1)

INFLOWS:
Stom_Secretion = PULSE(16.67,0,.1)

OUTFLOWS:
vol_sd = kf_sd*Vol_stom
Adsorp_Stom = 0*Vol_stom*ka_sd
conc_plasma =  (amt_plasma/volume)*mg_to_ug
k12 = .839
k21 = .67
ka_co = 1
ka_col = 3
ka_dj = 3
ka_du = 1
ka_ic = 3
ka_il = 8.83
ka_je = 1
ka_ji = 3
ka_sd = 1
kf_sd = 2.8
k_elim = .161
mg_to_ug = 1000
perm_colon = 3.80e-6
perm_duo = 1.10e-6
perm_Il = 4.06e-6
perm_jej = 2.17e-6
perm_stom = 1.10e-6
ph_s = 1.5
ph_s_2 = 6.6
ph_s_3 = 6.6
ph_s_4 = 7.5
ph_s_5 = 6.6
SA_colon = 138
SA_duo = 125
SA_Il = 102
SA_jej = 182
SA_stom = 50
volume = 4*19200
sol_profile = GRAPH(ph_s)
(1.00, 63.0), (1.50, 25.0), (2.00, 10.0), (2.50, 5.00), (3.00, 4.00), (3.50, 3.80), (4.00,
3.65), (4.50, 3.50), (5.00, 3.65), (5.50, 3.65), (6.00, 3.65), (6.50, 3.65), (7.00, 3.65),
(7.50, 3.65), (8.00, 3.65), (8.50, 4.00), (9.00, 5.00), (9.50, 12.0), (10.0, 23.5)
sol_profile_2 = GRAPH(ph_s_2)
```

```
(1.00, 63.0), (1.50, 25.0), (2.00, 10.0), (2.50, 5.00), (3.00, 4.00), (3.50, 3.80), (4.00,
3.65), (4.50, 3.50), (5.00, 3.65), (5.50, 3.65), (6.00, 3.65), (6.50, 3.65), (7.00, 3.65),
(7.50, 3.65), (8.00, 3.65), (8.50, 4.00), (9.00, 5.00), (9.50, 12.0), (10.0, 23.5)
sol_profile_3 = GRAPH(ph_s_3)
(1.00, 63.0), (1.50, 25.0), (2.00, 10.0), (2.50, 5.00), (3.00, 4.00), (3.50, 3.80), (4.00,
3.65), (4.50, 3.50), (5.00, 3.65), (5.50, 3.65), (6.00, 3.65), (6.50, 3.65), (7.00, 3.65),
(7.50, 3.65), (8.00, 3.65), (8.50, 4.00), (9.00, 5.00), (9.50, 12.0), (10.0, 23.5)
sol_profile_4 = GRAPH(ph_s_4)
(1.00, 63.0), (1.50, 25.0), (2.00, 10.0), (2.50, 5.00), (3.00, 4.00), (3.50, 3.80), (4.00,
3.65), (4.50, 3.50), (5.00, 3.65), (5.50, 3.65), (6.00, 3.65), (6.50, 3.65), (7.00, 3.65),
(7.50, 3.65), (8.00, 3.65), (8.50, 4.00), (9.00, 5.00), (9.50, 12.0), (10.0, 23.5)
sol_profile_5 = GRAPH(ph_s_5)
(1.00, 63.0), (1.50, 25.0), (2.00, 10.0), (2.50, 5.00), (3.00, 4.00), (3.50, 3.80), (4.00,
3.65), (4.50, 3.50), (5.00, 3.65), (5.50, 3.65), (6.00, 3.65), (6.50, 3.65), (7.00, 3.65),
(7.50, 3.65), (8.00, 3.65), (8.50, 4.00), (9.00, 5.00), (9.50, 12.0), (10.0, 23.5)
```

Appendix 3: Abbreviation Key For GI Model

The legend/key has been divided into sub-sections corresponding to the sub-sections of the model diagram.

Numbered suffixes (1, 2, 3, 4, 5, 6) have been assigned to distinguish between intestinal regions (stomach, duodenum, jejunum, ileum, colon, and waste, respectively).

1 – stomach
2 – duodenum
3 – jejunum
4 – ileum
5 – colon
6 – waste

For example, VOL 1 is the volume in the stomach, MASS 3 is the insoluble mass in the jejunum. In the equations, COMP 1 indicates the stomach, COMP 2 the duodenum, COMP 3, the jejunum, etc.

Ghosts are listed under the sub-section containing the original reservoir, flow regulator, or converter.

Abbreviations listed in italics are regionally dependent and set up as arrays to allow independent values for each intestinal region.

In general, ADJ as a prefix indicates a calculated parameter value (ADJ = adjusted), while ADJ as a suffix indicates an adjustment parameter (ADJ = adjustment).

Intestinal model

Reservoirs/Compartments

VOL ABS	Fluid volume absorbed
VOL	Fluid volume
C REL	Mass of drug contained with a formulation or controlled release device
MASS	Insoluble mass of drug (not contained within the formulation or controlled release device)
SOL	Soluble mass of drug
ABSORPTION	Mass of drug absorbed

Flow regulators

REABS	Rate of water absorption
VOL OUT	Fluid volume transit rate
CR OUT	Formulation or controlled release device transit rate

CR INPUT	Drug release rate from formulation or controlled release device
MASS OUT	Insoluble drug mass transit rate
DISS PRECIP	Dissolution rate
SOL OUT	Soluble drug mass transit rate
FLUX	Absorption rate

ADJ PARMS (Adjustment Parameters)

VOL ADJ	Fluid volume absorption adjustment parameter
DISS ADJ	Dissolution rate adjustment parameter
TRANSIT ADJ	Transit time adjustment parameter
SA ADJ	Surface area adjustment parameter
FLUX ADJ	Passive Absorption adjustment parameter
EFFLUX ADJ	Efflux or secretion adjustment parameter
CARRIER ADJ	Active absorption adjustment parameter

PARMS (Parameters)

VOL PARM	Fluid volume absorption rate constant
SURFACE AREA	Surface area available for absorption
DOSE	The administered dose of drug
INIT VOLUME	The administered volume of water or fluid
TIME IN HOURS	A clock
pH	The physiological pH value
PARACELLULAR	A user controlled switch used to adjust absorption based on absorption mechanism

TRANSIT TIME

TRANSFERS	GI transit rate constant
CUMU TT	Cumulative transit time
ADJ TRANSIT TIME	Adjusted GI transit time incorporating adjustment parameter and user input
USER TT INPUT	User controlled adjustments to the GI transit time

OUTPUT CALCULATIONS

ABSORBED TOTAL	Total mass of drug absorbed (sum of ABSORPTION 1...5)
FDp%	Fraction or the dose absorbed into portal vein x 100
FLUX TOTAL	Total absorption rate (sum of FLUX 1...5)
CUM DISS	Cumulative drug mass dissolved
CR Release	Cumulative drug mass released from formulation
CUM DISS RATE	Sum of DISS PRECIP 1...5

CR cumrate	Summ of CR INPUT 1...5

PERMEABILITY CALCULATION

ADJ PERM	Adjusted permeability incorporating all transport mechanisms and relevant adjustment parameters
ACT PE	Active or carrier-mediated absorptive permeability
Km	Constant from the Michaelis-Menten type permeability equation for active transport
REGIONAL	Passive permeability after regional correlation calculation (same as PASS PE if regional correlation is not used)
PASS PE	Passive permeability entered by user
RC	A logical function used in determining the regional correlation
RCSUM	A logical function used in determining the regional correlation

SOLUBILITY CALCULATION

USER pH	User supplied pH value for which a solubility value is available
USER SOLUB	User supplied solubility value corresponding to the USER pH value
ADJ SOLUB	Solubility calculated (if necessary) at the appropriate pH value using the entered USER pH and USER SOLUB values

CONTROLLED RELEASE CALCULATION

CR RATE	The instantaneous release rate from the formulation
CR DOSE	The total dose contained with the formulation
CR AT TIME	The cumulative drug mass release profile
CR AT LAST	The cumulative drug mass release profile

Note: CR AT TIME holds the value at the current time value (t), CR AT LAST holds the value at the immediately preceeding time value (t-1)

CONC CALCULATION

CONCENTRATIONS	The dissolved drug concentration

DISSOLUTION CALCULATION

PRECIP	Precipitation rate constant

DISSOL	Dissolution rate constant
ADJ DISS PRECIP	Adjusted rate constant incorporating PRECIP, DISSOL and calculated concentration

Appendix 4: Equations, Parameters and Values For GI Model

ADJ PARMS

○ CARRIER_ADJ[COMPS] = 0
○ DISS_ADJ[COMP_1] = 1
○ DISS_ADJ[COMP_2] = 1
○ DISS_ADJ[COMP_3] = 1
○ DISS_ADJ[COMP_4] = 1
○ DISS_ADJ[COMP_5] = 1
○ EFFLUX_ADJ[COMPS] = 1
○ FLUX_ADJ[COMP_1] = 1
○ FLUX_ADJ[COMP_2] = 10
○ FLUX_ADJ[COMP_3] = 8
○ FLUX_ADJ[COMP_4] = 2
○ FLUX_ADJ[COMP_5] = 1
○ SA_ADJ[COMP_1] = 1
○ SA_ADJ[COMP_2] = 1
○ SA_ADJ[COMP_3] = 1
○ SA_ADJ[COMP_4] = 1
○ SA_ADJ[COMP_5] = 1
○ TRANSIT_ADJ[COMP_1] = 1
○ TRANSIT_ADJ[COMP_2] = 1
○ TRANSIT_ADJ[COMP_3] = 1
○ TRANSIT_ADJ[COMP_4] = 1
○ TRANSIT_ADJ[COMP_5] = 1
○ VOL_ADJ[COMP_1] = 1
○ VOL_ADJ[COMP_2] = 1
○ VOL_ADJ[COMP_3] = 1
○ VOL_ADJ[COMP_4] = 1
○ VOL_ADJ[COMP_5] = 1

CONC CALCULATION

○ CONCENTRATIONS[COMP_1] = if VOL_1=0.0 then 0 else if ADJ_SOLUB[COMP_1]<SOL_1/VOL_1 then ADJ_SOLUB[COMP_1] else SOL_1/VOL_1 + 0*(SOL_2+SOL_5+SOL_3+SOL_4+VOL_3+VOL_2+VOL_4+VOL_5)

○ CONCENTRATIONS[COMP_2] = if VOL_2 = 0.0 then 0 else if (VOL_2<1e-6 AND SOL_2<1e-7) then 0 else if ADJ_SOLUB[COMP_2]<SOL_2/VOL_2 then ADJ_SOLUB[COMP_2] else SOL_2/VOL_2
+0*(SOL_1+SOL_5+SOL_3+SOL_4+VOL_3+VOL_1+VOL_5+VOL_4)

○ CONCENTRATIONS[COMP_3] = if VOL_3 = 0.0 then 0 else if (VOL_3<1e-6 AND SOL_3<1e-7) then 0 else if ADJ_SOLUB[COMP_3]<SOL_3/VOL_3 then ADJ_SOLUB[COMP_3] else SOL_3/VOL_3
+0*(SOL_1+SOL_2+SOL_4+SOL_5+VOL_5+VOL_4+VOL_1+VOL_2)

○ CONCENTRATIONS[COMP_4] = if VOL_4 = 0.0 then 0 else if (VOL_4<1e-6 AND SOL_4<1e-7) then 0 else if ADJ_SOLUB[COMP_4]<SOL_4/VOL_4 then ADJ_SOLUB[COMP_4] else SOL_4/VOL_4
+0*(SOL_1+SOL_2+SOL_3+SOL_5+VOL_1+VOL_2+VOL_3+VOL_5)

○ CONCENTRATIONS[COMP_5] = if VOL_5 = 0.0 then 0 else if (VOL_5<1e-6 AND SOL_5<1e-7) then 0 else if ADJ_SOLUB[COMP_5]<SOL_5/VOL_5 then ADJ_SOLUB[COMP_5] else SOL_5/VOL_5
+0*(SOL_1+SOL_4+SOL_3+SOL_2+VOL_3+VOL_1+VOL_2+VOL_4)

▣ CONTROL RELEASE CALCULATION

○ CR_DOSE = 0

○ CR_RATE = (CR_AT_TIME-CR_AT_LAST)*20*(CR_DOSE/100)

○ CR_AT_LAST = GRAPH(TIME-DT)
(0.00, 0.00), (0.25, 17.7), (0.5, 31.5), (0.75, 42.2), (1.00, 50.6), (1.25, 57.1), (1.50, 62.1), (1.75, 66.1), (2.00, 69.2), (2.25, 71.6), (2.50, 73.4), (2.75, 74.9), (3.00, 76.0), (3.25, 76.9), (3.50, 77.6), (3.75, 78.1), (4.00, 78.5), (4.25, 78.9), (4.50, 79.1), (4.75, 79.3), (5.00, 79.5), (5.25, 79.6), (5.50, 79.7), (5.75, 79.7), (6.00, 79.8), (6.25, 79.8), (6.50, 79.9), (6.75, 79.9), (7.00, 79.9), (7.25, 79.9), (7.50, 80.0), (7.75, 80.0), (8.00, 80.0), (8.25, 80.0), (8.50, 80.0), (8.75, 80.0), (9.00, 80.0), (9.25, 80.0), (9.50, 80.0), (9.75, 80.0), (10.0, 80.0), (10.3, 80.0), (10.5, 80.0), (10.8, 80.0), (11.0, 80.0), (11.3, 80.0), (11.5, 80.0), (11.8, 80.0), (12.0, 80.0), (12.3, 80.0), (12.5, 80.0), (12.8, 80.0), (13.0, 80.0)...

○ CR_AT_TIME = GRAPH(TIME)
(0.00, 0.00), (0.25, 17.7), (0.5, 31.5), (0.75, 42.2), (1.00, 50.6), (1.25, 57.1), (1.50, 62.1), (1.75, 66.1), (2.00, 69.2), (2.25, 71.6), (2.50, 73.4), (2.75, 74.9), (3.00, 76.0), (3.25, 76.9), (3.50, 77.6), (3.75, 78.1), (4.00, 78.5), (4.25, 78.9), (4.50, 79.1), (4.75, 79.3), (5.00, 79.5), (5.25, 79.6), (5.50, 79.7), (5.75, 79.7), (6.00, 79.8), (6.25, 79.8), (6.50, 79.9), (6.75, 79.9), (7.00, 79.9), (7.25, 79.9), (7.50, 80.0), (7.75, 80.0), (8.00, 80.0), (8.25, 80.0), (8.50, 80.0), (8.75, 80.0), (9.00, 80.0), (9.25, 80.0), (9.50, 80.0), (9.75, 80.0), (10.0, 80.0), (10.3, 80.0), (10.5, 80.0), (10.8, 80.0), (11.0, 80.0), (11.3, 80.0), (11.5, 80.0), (11.8, 80.0), (12.0, 80.0), (12.3, 80.0), (12.5, 80.0), (12.8, 80.0), (13.0, 80.0)...

▣ DISSOLUTION CALCULATION

○ ADJ_DISS_PRECIP[COMP_1] = if VOL_1=0 then 0 else if (SOL_1/VOL_1<ADJ_SOLUB[COMP_1]) then (DISSOL[COMP_1]*DISS_ADJ[COMP_1]*MASS_1*(ADJ_SOLUB[COMP_1]-SOL_1/VOL_1)) else ((SOL_1/VOL_1)-ADJ_SOLUB[COMP_1])*PRECIP[COMP_1]+
0*(MASS_1+MASS_2+MASS_3+MASS_4+MASS_5+SOL_1+SOL_2+SOL_3+SOL_4+SOL_5+VOL_1+VOL_2+VOL_3+VOL_4+VOL_5)

○ ADJ_DISS_PRECIP[COMP_2] = if VOL_2=0 then 0 else if (SOL_2/VOL_2<ADJ_SOLUB[COMP_2]) then (DISSOL[COMP_2]*DISS_ADJ[COMP_2]*MASS_2*(ADJ_SOLUB[COMP_2]-SOL_2/VOL_2)) else ((SOL_2/VOL_2)-ADJ_SOLUB[COMP_2])*PRECIP[COMP_2]
+0*(MASS_1+MASS_2+MASS_3+MASS_4+MASS_5+SOL_1+SOL_2+SOL_3+SOL_4+SOL_5+VOL_1+VOL_2+VOL_3+VOL_4+VOL_5)

○ ADJ_DISS_PRECIP[COMP_3] = if VOL_3=0 then 0 else if (SOL_3/VOL_3<ADJ_SOLUB[COMP_3]) then (DISSOL[COMP_3]*DISS_ADJ[COMP_3]*MASS_3*(ADJ_SOLUB[COMP_3]-SOL_3/VOL_3)) else ((SOL_3/VOL_3)-ADJ_SOLUB[COMP_3])*PRECIP[COMP_3]
+0*(MASS_1+MASS_2+MASS_3+MASS_4+MASS_5+SOL_1+SOL_2+SOL_3+SOL_4+SOL_5+VOL_1+VOL_2+VOL_3+VOL_4+VOL_5)

```
○ ADJ_DISS_PRECIP[COMP_4] = if VOL_4=0 then 0 else if
  (SOL_4/VOL_4<ADJ_SOLUB[COMP_4]) then
  (DISSOL[COMP_4]*DISS_ADJ[COMP_4]*MASS_4*(ADJ_SOLUB[COMP_4]-SOL_4/VOL_4)) else
  ((SOL_4/VOL_4)-ADJ_SOLUB[COMP_4])*PRECIP[COMP_4]
  +0*(MASS_1+MASS_2+MASS_3+MASS_4+MASS_5+SOL_1+SOL_2+SOL_3+SOL_4+SOL_5+V
  OL_1+VOL_2+VOL_3+VOL_4+VOL_5)
○ ADJ_DISS_PRECIP[COMP_5] = if VOL_5=0 then 0 else if
  (SOL_5/VOL_5<ADJ_SOLUB[COMP_5]) then
  (DISSOL[COMP_5]*DISS_ADJ[COMP_5]*MASS_5*(ADJ_SOLUB[COMP_5]-SOL_5/VOL_5)) else
  ((SOL_5/VOL_5)-ADJ_SOLUB[COMP_5])*PRECIP[COMP_5]
  +0*(MASS_1+MASS_2+MASS_3+MASS_4+MASS_5+SOL_1+SOL_2+SOL_3+SOL_4+SOL_5+V
  OL_1+VOL_2+VOL_3+VOL_4+VOL_5)
○ DISSOL[COMP_1] = 1
○ DISSOL[COMP_2] = 1
○ DISSOL[COMP_3] = 1
○ DISSOL[COMP_4] = 1
○ DISSOL[COMP_5] = 1
○ PRECIP[COMP_1] = 10
○ PRECIP[COMP_2] = 10
○ PRECIP[COMP_3] = 10
○ PRECIP[COMP_4] = 10
○ PRECIP[COMP_5] = 10
INPUTS
INTESTINAL MODEL
☐ ABSORPTION_1(t) = ABSORPTION_1(t - dt) + (FLUX_1) * dt
  INIT ABSORPTION_1 = 0
   INFLOWS:
      FLUX_1 =
      CONCENTRATIONS[COMP_1]*ADJ_PERM[COMP_1]*SURFACE_AREA[COMP_1]

☐ ABSORPTION_2(t) = ABSORPTION_2(t - dt) + (FLUX_2) * dt
  INIT ABSORPTION_2 = 0
   INFLOWS:
      FLUX_2 =
      CONCENTRATIONS[COMP_2]*ADJ_PERM[COMP_2]*SURFACE_AREA[COMP_2]

☐ ABSORPTION_3(t) = ABSORPTION_3(t - dt) + (FLUX_3) * dt
  INIT ABSORPTION_3 = 0
   INFLOWS:
      FLUX_3 =
      CONCENTRATIONS[COMP_3]*ADJ_PERM[COMP_3]*SURFACE_AREA[COMP_3]

☐ ABSORPTION_4(t) = ABSORPTION_4(t - dt) + (FLUX_4) * dt
  INIT ABSORPTION_4 = 0
   INFLOWS:
        FLUX_4 =
        CONCENTRATIONS[COMP_4]*ADJ_PERM[COMP_4]*SURFACE_AREA[COMP_4]

    ABSORPTION_5(t) = ABSORPTION_5(t - dt) + (FLUX_5) * dt
    INIT ABSORPTION_5 = 0
     INFLOWS:
        FLUX_5 = if time<32 then
        CONCENTRATIONS[COMP_5]*ADJ_PERM[COMP_5]*SURFACE_AREA[COMP_5]*(32-ti
        me)/48*(VOL_5/17.2) else 0
    C_REL_1(t) = C_REL_1(t - dt) + (- CR_OUT_1 - CR_INPUT_1) * dt
    INIT C_REL_1 = CR_DOSE
     OUTFLOWS:
        CR_OUT_1 = IF TIME >=  CUMU_TT[COMP_1] THEN C_REL_1*10000 ELSE 0
        CR_INPUT_1 = if TIME>CUMU_TT[COMP_1] then 0 else CR_RATE
    C_REL_2(t) = C_REL_2(t - dt) + (CR_OUT_1 - CR_OUT_2 - CR_INPUT_2) * dt
    INIT C_REL_2 = 0
     INFLOWS:
        CR_OUT_1 = IF TIME >=  CUMU_TT[COMP_1] THEN C_REL_1*10000 ELSE 0
     OUTFLOWS:
        CR_OUT_2 = IF TIME >=  CUMU_TT[COMP_2] THEN C_REL_2*10000 ELSE 0
        CR_INPUT_2 = if TIME>CUMU_TT[COMP_2] then 0 else CR_RATE
    C_REL_3(t) = C_REL_3(t - dt) + (CR_OUT_2 - CR_OUT_3 - CR_INPUT_3) * dt
    INIT C_REL_3 = 0
     INFLOWS:
        CR_OUT_2 = IF TIME >=  CUMU_TT[COMP_2] THEN C_REL_2*10000 ELSE 0
     OUTFLOWS:
        CR_OUT_3 = IF TIME >=  CUMU_TT[COMP_3] THEN C_REL_3*10000 ELSE 0
        CR_INPUT_3 = if TIME > CUMU_TT[COMP_3] then 0 else CR_RATE
    C_REL_4(t) = C_REL_4(t - dt) + (CR_OUT_3 - CR_OUT_4 - CR_INPUT_4) * dt
    INIT C_REL_4 = 0
     INFLOWS:
        CR_OUT_3 = IF TIME >=  CUMU_TT[COMP_3] THEN C_REL_3*10000 ELSE 0
     OUTFLOWS:
        CR_OUT_4 = IF TIME >=  CUMU_TT[COMP_4] THEN C_REL_4*10000 ELSE 0
        CR_INPUT_4 = if TIME>CUMU_TT[COMP_4] then 0 else CR_RATE
    C_REL_5(t) = C_REL_5(t - dt) + (CR_OUT_4 - CR_OUT_5 - CR_INPUT_5) * dt
    INIT C_REL_5 = 0
     INFLOWS:
        CR_OUT_4 = IF TIME >=  CUMU_TT[COMP_4] THEN C_REL_4*10000 ELSE 0
     OUTFLOWS:
        CR_OUT_5 = IF TIME >=  CUMU_TT[COMP_5] THEN C_REL_5*10000 ELSE 0
        CR_INPUT_5 = if TIME>CUMU_TT[COMP_5] then 0 else CR_RATE
    C_REL_6(t) = C_REL_6(t - dt) + (CR_OUT_5) * dt
    INIT C_REL_6 = 0
     INFLOWS:
        CR_OUT_5 = IF TIME >=  CUMU_TT[COMP_5] THEN C_REL_5*10000 ELSE 0
```

```
☐ MASS_1(t) = MASS_1(t - dt) + (CR_INPUT_1 - MASS_OUT_1 - DISS_PRECIP_1) * dt
  INIT MASS_1 = DOSE
   INFLOWS:
      CR_INPUT_1 = if TIME>CUMU_TT[COMP_1] then 0 else CR_RATE
   OUTFLOWS:
      MASS_OUT_1 = MASS_1*TRANSFERS[COMP_1]
      DISS_PRECIP_1 = ADJ_DISS_PRECIP[COMP_1]
☐ MASS_2(t) = MASS_2(t - dt) + (MASS_OUT_1 + CR_INPUT_2 - MASS_OUT_2 -
  DISS_PRECIP_2) * dt
  INIT MASS_2 = 0
   INFLOWS:
      MASS_OUT_1 = MASS_1*TRANSFERS[COMP_1]
      CR_INPUT_2 = if TIME>CUMU_TT[COMP_2] then 0 else CR_RATE
   OUTFLOWS:
      MASS_OUT_2 = MASS_2*TRANSFERS[COMP_2]
      DISS_PRECIP_2 = ADJ_DISS_PRECIP[COMP_2]
☐ MASS_3(t) = MASS_3(t - dt) + (CR_INPUT_3 + MASS_OUT_2 - MASS_OUT_3 -
  DISS_PRECIP_3) * dt
  INIT MASS_3 = 0
   INFLOWS:
      CR_INPUT_3 = if TIME > CUMU_TT[COMP_3] then 0 else CR_RATE
      MASS_OUT_2 = MASS_2*TRANSFERS[COMP_2]
   OUTFLOWS:
      MASS_OUT_3 = MASS_3*TRANSFERS[COMP_3]
      DISS_PRECIP_3 = ADJ_DISS_PRECIP[COMP_3]
☐ MASS_4(t) = MASS_4(t - dt) + (CR_INPUT_4 + MASS_OUT_3 - MASS_OUT_4 -
  DISS_PRECIP_4) * dt
  INIT MASS_4 = 0
   INFLOWS:
      CR_INPUT_4 = if TIME>CUMU_TT[COMP_4] then 0 else CR_RATE
      MASS_OUT_3 = MASS_3*TRANSFERS[COMP_3]
   OUTFLOWS:
      MASS_OUT_4 = MASS_4*TRANSFERS[COMP_4]
      DISS_PRECIP_4 = ADJ_DISS_PRECIP[COMP_4]
☐ MASS_5(t) = MASS_5(t - dt) + (CR_INPUT_5 + MASS_OUT_4 - MASS_OUT_5 -
  DISS_PRECIP_5) * dt
  INIT MASS_5 = 0
   INFLOWS:
      CR_INPUT_5 = if TIME>CUMU_TT[COMP_5] then 0 else CR_RATE
      MASS_OUT_4 = MASS_4*TRANSFERS[COMP_4]
   OUTFLOWS:
      MASS_OUT_5 = if time>4 then MASS_5*TRANSFERS[COMP_5] else 0
      DISS_PRECIP_5 = ADJ_DISS_PRECIP[COMP_5]
☐ MASS_6(t) = MASS_6(t - dt) + (MASS_OUT_5) * dt
  INIT MASS_6 = 0
   INFLOWS:
      MASS_OUT_5 = if time>4 then MASS_5*TRANSFERS[COMP_5] else 0
☐ SOL_1(t) = SOL_1(t - dt) + (DISS_PRECIP_1 - SOL_OUT_1 - FLUX_1) * dt
  INIT SOL_1 = 0
   INFLOWS:
      DISS_PRECIP_1 = ADJ_DISS_PRECIP[COMP_1]
   OUTFLOWS:
      SOL_OUT_1 = SOL_1*TRANSFERS[COMP_1]
      FLUX_1 =
      CONCENTRATIONS[COMP_1]*ADJ_PERM[COMP_1]*SURFACE_AREA[COMP_1]

☐ SOL_2(t) = SOL_2(t - dt) + (SOL_OUT_1 + DISS_PRECIP_2 - SOL_OUT_2 - FLUX_2) * dt
  INIT SOL_2 = 0

   INFLOWS:
      SOL_OUT_1 = SOL_1*TRANSFERS[COMP_1]
      DISS_PRECIP_2 = ADJ_DISS_PRECIP[COMP_2]
   OUTFLOWS:
      SOL_OUT_2 = SOL_2*TRANSFERS[COMP_2]
      FLUX_2 =
      CONCENTRATIONS[COMP_2]*ADJ_PERM[COMP_2]*SURFACE_AREA[COMP_2]

☐ SOL_3(t) = SOL_3(t - dt) + (DISS_PRECIP_3 + SOL_OUT_2 - SOL_OUT_3 - FLUX_3) * dt
  INIT SOL_3 = 0

   INFLOWS:
      DISS_PRECIP_3 = ADJ_DISS_PRECIP[COMP_3]
      SOL_OUT_2 = SOL_2*TRANSFERS[COMP_2]
   OUTFLOWS:
      SOL_OUT_3 = SOL_3*TRANSFERS[COMP_3]
      FLUX_3 =
      CONCENTRATIONS[COMP_3]*ADJ_PERM[COMP_3]*SURFACE_AREA[COMP_3]

☐ SOL_4(t) = SOL_4(t - dt) + (DISS_PRECIP_4 + SOL_OUT_3 - SOL_OUT_4 - FLUX_4) * dt
  INIT SOL_4 = 0

   INFLOWS:
      DISS_PRECIP_4 = ADJ_DISS_PRECIP[COMP_4]
      SOL_OUT_3 = SOL_3*TRANSFERS[COMP_3]
   OUTFLOWS:
      SOL_OUT_4 = SOL_4*TRANSFERS[COMP_4]
      FLUX_4 =
      CONCENTRATIONS[COMP_4]*ADJ_PERM[COMP_4]*SURFACE_AREA[COMP_4]

☐ SOL_5(t) = SOL_5(t - dt) + (DISS_PRECIP_5 + SOL_OUT_4 - SOL_OUT_5 - FLUX_5) * dt
  INIT SOL_5 = 0

   INFLOWS:
      DISS_PRECIP_5 = ADJ_DISS_PRECIP[COMP_5]
      SOL_OUT_4 = SOL_4*TRANSFERS[COMP_4]
    OUTFLOWS:
      SOL_OUT_5 = if time>4 then SOL_5*TRANSFERS[COMP_5] else 0
      FLUX_5 = if time<32 then
      CONCENTRATIONS[COMP_5]*ADJ_PERM[COMP_5]*SURFACE_AREA[COMP_5]*(32-ti
      me)/48*(VOL_5/17.2) else 0
☐ SOL_6(t) = SOL_6(t - dt) + (SOL_OUT_5) * dt
   INIT SOL_6 = 0
    INFLOWS:
      SOL_OUT_5 = if time>4 then SOL_5*TRANSFERS[COMP_5] else 0
☐ VOL_1(t) = VOL_1(t - dt) + (- REABS_1 - VOL_OUT_1) * dt
   INIT VOL_1 = INIT_VOLUME
    OUTFLOWS:
      REABS_1 = VOL_1*VOL_PARM[COMP_1]
      VOL_OUT_1 = VOL_1*TRANSFERS[COMP_1]
☐ VOL_2(t) = VOL_2(t - dt) + (VOL_OUT_1 - VOL_OUT_2 - REABS_2) * dt
   INIT VOL_2 = 0
    INFLOWS:
      VOL_OUT_1 = VOL_1*TRANSFERS[COMP_1]
    OUTFLOWS:
      VOL_OUT_2 = VOL_2*TRANSFERS[COMP_2]
      REABS_2 = VOL_2*VOL_PARM[COMP_2]
☐ VOL_3(t) = VOL_3(t - dt) + (VOL_OUT_2 - VOL_OUT_3 - REABS_3) * dt
   INIT VOL_3 = 0
    INFLOWS:
      VOL_OUT_2 = VOL_2*TRANSFERS[COMP_2]
    OUTFLOWS:
      VOL_OUT_3 = VOL_3*TRANSFERS[COMP_3]
      REABS_3 = VOL_3*VOL_PARM[COMP_3]
☐ VOL_4(t) = VOL_4(t - dt) + (VOL_OUT_3 - VOL_OUT_4 - REABS_4) * dt
   INIT VOL_4 = 0
    INFLOWS:
      VOL_OUT_3 = VOL_3*TRANSFERS[COMP_3]
    OUTFLOWS:
      VOL_OUT_4 = VOL_4*TRANSFERS[COMP_4]
      REABS_4 = VOL_4*VOL_PARM[COMP_4]
☐ VOL_5(t) = VOL_5(t - dt) + (VOL_OUT_4 - VOL_OUT_5 - REABS_5) * dt
   INIT VOL_5 = 0
    INFLOWS:
      VOL_OUT_4 = VOL_4*TRANSFERS[COMP_4]
    OUTFLOWS:
      VOL_OUT_5 = VOL_5*TRANSFERS[COMP_5]
      REABS_5 = VOL_5*VOL_PARM[COMP_5]
☐ VOL_6(t) = VOL_6(t - dt) + (VOL_OUT_5) * dt
   INIT VOL_6 = 0
```

```
      INFLOWS:
        ⇸ VOL_OUT_5 = VOL_5*TRANSFERS[COMP_5]
  ☐ VOL_ABS_1(t) = VOL_ABS_1(t - dt) + (REABS_1) * dt
     INIT VOL_ABS_1 = 0
      INFLOWS:
        ⇸ REABS_1 = VOL_1*VOL_PARM[COMP_1]
  ☐ VOL_ABS_2(t) = VOL_ABS_2(t - dt) + (REABS_2) * dt
     INIT VOL_ABS_2 = 0
      INFLOWS:
        ⇸ REABS_2 = VOL_2*VOL_PARM[COMP_2]
  ☐ VOL_ABS_3(t) = VOL_ABS_3(t - dt) + (REABS_3) * dt
     INIT VOL_ABS_3 = 0
      INFLOWS:
        ⇸ REABS_3 = VOL_3*VOL_PARM[COMP_3]
  ☐ VOL_ABS_4(t) = VOL_ABS_4(t - dt) + (REABS_4) * dt
     INIT VOL_ABS_4 = 0
      INFLOWS:
        ⇸ REABS_4 = VOL_4*VOL_PARM[COMP_4]
  ☐ VOL_ABS_5(t) = VOL_ABS_5(t - dt) + (REABS_5) * dt
     INIT VOL_ABS_5 = 0
      INFLOWS:
        ⇸ REABS_5 = VOL_5*VOL_PARM[COMP_5]
▣ MULTI DOSE CALCULATION
▣ OUTPUT CALCULATIONS
  ☐ CR_Release(t) = CR_Release(t - dt) + (CR_cumrate) * dt
     INIT CR_Release = 0
      INFLOWS:
        ⇸ CR_cumrate = CR_INPUT_1+CR_INPUT_2+CR_INPUT_3+CR_INPUT_4+CR_INPUT_5

  ☐ CUM_DISS(t) = CUM_DISS(t - dt) + (CUMM_DISS_RATE) * dt
     INIT CUM_DISS = 0
      INFLOWS:
        ⇸ CUMM_DISS_RATE =
          DISS_PRECIP_1+DISS_PRECIP_2+DISS_PRECIP_3+DISS_PRECIP_4+DISS_PRECIP
          5
  ○ ABSORBED_TOTAL = ABSORPTION_2+ABSORPTION_3+ABSORPTION_4+ABSORPTION_5

  ○ FDp% = ABSORBED_TOTAL/DOSE*100
  ○ FLUX_TOTAL = FLUX_2+FLUX_3+FLUX_4+FLUX_5
▣ PARMS
  ○ DOSE = 1000
  ○ INIT_VOLUME = 100
  ○ PARACELLULAR = 1
  ○ pH[COMP_1] = 1.5
  ○ pH[COMP_2] = 5
  ○ pH[COMP_3] = 6.5
○ pH[COMP_4] = 7
○ pH[COMP_5] = 6.5
○ SURFACE_AREA[COMP_1] = if PARACELLULAR =0 then 50*SA_ADJ[COMP_1] else
  50*SA_ADJ[COMP_1]
○ SURFACE_AREA[COMP_2] = if PARACELLULAR=0 then 150*SA_ADJ[COMP_2] else
  7.5*SA_ADJ[COMP_2]
○ SURFACE_AREA[COMP_3] = if PARACELLULAR=0 then 1000*SA_ADJ[COMP_3] else
  50*SA_ADJ[COMP_3]
○ SURFACE_AREA[COMP_4] = if PARACELLULAR=0 then 1000*SA_ADJ[COMP_4] else
  50*SA_ADJ[COMP_4]
○ SURFACE_AREA[COMP_5] = if PARACELLULAR=0 then 850*SA_ADJ[COMP_5] else
  42.5*SA_ADJ[COMP_5]
○ TIME_IN_HOURS = TIME
○ VOL_PARM[COMP_1] = 0*VOL_ADJ[COMP_1]
○ VOL_PARM[COMP_2] = 0*VOL_ADJ[COMP_2]
○ VOL_PARM[COMP_3] = 1.75*VOL_ADJ[COMP_3]
○ VOL_PARM[COMP_4] = 1.75*VOL_ADJ[COMP_4]
○ VOL_PARM[COMP_5] = 0.10*VOL_ADJ[COMP_5]
▭ PERMEABILITY CALCULATION
○ ACT_PE[COMPS] = [0 ,
  0 ,
  0 ,
  0 ,
  0 ]
○ ADJ_PERM[COMP_1] =
  (2/(1+EFFLUX_ADJ[COMP_1]))*REGIONAL[COMP_1]*FLUX_ADJ[COMP_1]*3600+(CARRIER_
  DJ[COMP_1]*ACT_PE[COMP_1]*3600/(1+(CONCENTRATIONS[COMP_1]/(Km[COMP_1]))))*0

○ ADJ_PERM[COMP_2] =
  (2/(1+EFFLUX_ADJ[COMP_2]))*REGIONAL[COMP_2]*FLUX_ADJ[COMP_2]*3600+(CARRIER_
  DJ[COMP_2]*ACT_PE[COMP_2]*3600/(1+(CONCENTRATIONS[COMP_2]/(Km[COMP_2]))))

○ ADJ_PERM[COMP_3] =
  (2/(1+EFFLUX_ADJ[COMP_3]))*REGIONAL[COMP_3]*FLUX_ADJ[COMP_3]*3600+(CARRIER_
  DJ[COMP_3]*ACT_PE[COMP_3]*3600/(1+(CONCENTRATIONS[COMP_3]/(Km[COMP_3]))))

○ ADJ_PERM[COMP_4] =
  (2/(1+EFFLUX_ADJ[COMP_4]))*REGIONAL[COMP_4]*FLUX_ADJ[COMP_4]*3600+(CARRIER_
  DJ[COMP_4]*ACT_PE[COMP_4]*3600/(1+(CONCENTRATIONS[COMP_4]/(Km[COMP_4]))))

○ ADJ_PERM[COMP_5] =
  (2/(1+EFFLUX_ADJ[COMP_5]))*REGIONAL[COMP_5]*FLUX_ADJ[COMP_5]*3600+(CARRIER_
  DJ[COMP_5]*ACT_PE[COMP_5]*3600/(1+(CONCENTRATIONS[COMP_5]/(Km[COMP_5]))))
```

```
○ Km[COMPS] = [1 ,
  1 ,
  1 ,
  1 ,
  1 ]
○ PASS_PE[COMPS] = [0 ,
  1.10E-06 ,
  2.17E-06 ,
  4.06E-06 ,
  3.80E-06 ]
○ RC[COMP_1] = PASS_PE[COMP_1]*0
○ RC[COMP_2] = IF PASS_PE[COMP_2]>0 THEN 1 ELSE 0
○ RC[COMP_3] = IF PASS_PE[COMP_3]>0 THEN 2 ELSE 0
○ RC[COMP_4] = IF PASS_PE[COMP_4]>0 THEN 4 ELSE 0
○ RC[COMP_5] = PASS_PE[COMP_5]*0
○ RCSUM = RC[COMP_2]+RC[COMP_3]+RC[COMP_4]
○ REGIONAL[COMP_1] = PASS_PE[COMP_1]+RCSUM*0
○ REGIONAL[COMP_2] = if RCSUM=2 then
  (EXP( -9.011926 + 2.594378 *LOGN(1/PASS_PE[COMP_2]) -0.065515
  *LOGN(1/PASS_PE[COMP_2])^2))^(-1) else
  if RCSUM=4 then

  (EXP(-0.369414*LOGN(1/PASS_PE[COMP_4])+0.23756*LOGN(1/PASS_PE[COMP_4])^2-0.009
  9719*LOGN(1/PASS_PE[COMP_4])^3))^(-1) else
  if RCSUM=6 then
  0.5*(EXP( -9.011926 + 2.594378 *LOGN(1/PASS_PE[COMP_3]) -0.065515
  *LOGN(1/PASS_PE[COMP_3])^2))^(-1)
  +0.5*(EXP( -21.009845 + 4.544238 *LOGN(1/PASS_PE[COMP_4]) -0.140815
  *LOGN(1/PASS_PE[COMP_4])^2))^(-1) else
  PASS_PE[COMP_2]
○ REGIONAL[COMP_3] = if RCSUM=1 then
  (EXP( -3.238469 + 1.509131 *LOGN(1/PASS_PE[COMP_2]) -0.022109
  *LOGN(1/PASS_PE[COMP_2])^2))^(-1) else
  if RCSUM=4 then

  (EXP(-0.093739*LOGN(1/PASS_PE[COMP_4])+0.182344*LOGN(1/PASS_PE[COMP_4])^2-0.00
  23631*LOGN(1/PASS_PE[COMP_4])^3))^(-1) else
  if RCSUM=5 then
  0.5*(EXP( -3.238469 + 1.509131 *LOGN(1/PASS_PE[COMP_2]) -0.022109
  *LOGN(1/PASS_PE[COMP_2])^2))^(-1)
  +0.5*(EXP( -15.415683 + 3.543563 *LOGN(1/PASS_PE[COMP_4]) -0.100318
  *LOGN(1/PASS_PE[COMP_4])^2))^(-1) else
  PASS_PE[COMP_3]
```

```
○ REGIONAL[COMP_4] = if RCSUM=1 then
  (EXP( 14.455255 -1.264630 *LOGN(1/PASS_PE[COMP_2]) + 0.082015
  *LOGN(1/PASS_PE[COMP_2])^2))^(-1) else
  if RCSUM=2 then
  (EXP( 11.480333 -0.791109 *LOGN(1/PASS_PE[COMP_3]) + 0.066063
  *LOGN(1/PASS_PE[COMP_3])^2))^(-1) else
  if RCSUM=3 then
  0.5*(EXP( 14.455255 -1.264630 *LOGN(1/PASS_PE[COMP_2]) + 0.082015
  *LOGN(1/PASS_PE[COMP_2])^2))^(-1)
  +0.5*(EXP( 11.480333 -0.791109 *LOGN(1/PASS_PE[COMP_3]) + 0.066063
  *LOGN(1/PASS_PE[COMP_3])^2))^(-1) else
  PASS_PE[COMP_4]
○ REGIONAL[COMP_5] = PASS_PE[COMP_5] +RCSUM*0
▣ SOLUBILIY CALCULATION
○ ADJ_SOLUB[COMP_1] = if USER_pH[COMP_1]>=pH[COMP_1] then USER_SOLUB[COMP_1]
  else
  ((USER_SOLUB[COMP_2]-USER_SOLUB[COMP_1])/(USER_pH[COMP_2]-USER_pH[COMP_1]
  ))*(pH[COMP_1]-USER_pH[COMP_1])+USER_SOLUB[COMP_1]
○ ADJ_SOLUB[COMP_2] = if USER_pH[COMP_2]=pH[COMP_2] then USER_SOLUB[COMP_2]
  else if USER_pH[COMP_2]<pH[COMP_2] then
  ((USER_SOLUB[COMP_3]-USER_SOLUB[COMP_2])/(USER_pH[COMP_3]-USER_pH[COMP_2]
  ))*(pH[COMP_2]-USER_pH[COMP_2])+USER_SOLUB[COMP_2] else
  ((USER_SOLUB[COMP_2]-USER_SOLUB[COMP_1])/(USER_pH[COMP_2]-USER_pH[COMP_1]
  ))*(pH[COMP_2]-USER_pH[COMP_1])+USER_SOLUB[COMP_1]
○ ADJ_SOLUB[COMP_3] = if USER_pH[COMP_3]=pH[COMP_3] then USER_SOLUB[COMP_3]
  else if USER_pH[COMP_3]<pH[COMP_3] then
  ((USER_SOLUB[COMP_4]-USER_SOLUB[COMP_3])/(USER_pH[COMP_4]-USER_pH[COMP_3]
  ))*(pH[COMP_3]-USER_pH[COMP_3])+USER_SOLUB[COMP_3] else
  ((USER_SOLUB[COMP_3]-USER_SOLUB[COMP_2])/(USER_pH[COMP_3]-USER_pH[COMP_2]
  ))*(pH[COMP_3]-USER_pH[COMP_2])+USER_SOLUB[COMP_2]
○ ADJ_SOLUB[COMP_4] = if USER_pH[COMP_4]=pH[COMP_4] then USER_SOLUB[COMP_4]
  else if USER_pH[COMP_4]<pH[COMP_4] then
  ((USER_SOLUB[COMP_5]-USER_SOLUB[COMP_4])/(USER_pH[COMP_5]-USER_pH[COMP_4]
  ))*(pH[COMP_4]-USER_pH[COMP_4])+USER_SOLUB[COMP_4] else
  ((USER_SOLUB[COMP_4]-USER_SOLUB[COMP_3])/(USER_pH[COMP_4]-USER_pH[COMP_3]
  ))*(pH[COMP_4]-USER_pH[COMP_3])+USER_SOLUB[COMP_3]
○ ADJ_SOLUB[COMP_5] = if USER_pH[COMP_3]=pH[COMP_3] then USER_SOLUB[COMP_3]
  else if USER_pH[COMP_3]<pH[COMP_3] then
  ((USER_SOLUB[COMP_4]-USER_SOLUB[COMP_3])/(USER_pH[COMP_4]-USER_pH[COMP_3]
  ))*(pH[COMP_3]-USER_pH[COMP_3])+USER_SOLUB[COMP_3] else
  ((USER_SOLUB[COMP_3]-USER_SOLUB[COMP_2])/(USER_pH[COMP_3]-USER_pH[COMP_2]
  ))*(pH[COMP_3]-USER_pH[COMP_2])+USER_SOLUB[COMP_2]
○ USER_pH[COMPS] = [1.5 ,
  5 ,
  6.5 ,
  7 ,
  7.5 ]
  ○ USER_SOLUB[COMPS] = [31 ,
    3.65 ,
    3.65 ,
    3.65 ,
    3.65 ]
▣ TRANSIT TIME
  ○ ADJ_TRANSIT_TIME[COMP_1] = .5*TRANSIT_ADJ[COMP_1]*USER_TT_INPUT
  ○ ADJ_TRANSIT_TIME[COMP_2] = .25*TRANSIT_ADJ[COMP_2]*USER_TT_INPUT
  ○ ADJ_TRANSIT_TIME[COMP_3] = 1.5*TRANSIT_ADJ[COMP_3]*USER_TT_INPUT
  ○ ADJ_TRANSIT_TIME[COMP_4] = 1.5*TRANSIT_ADJ[COMP_4]*USER_TT_INPUT
  ○ ADJ_TRANSIT_TIME[COMP_5] = 24*TRANSIT_ADJ[COMP_5]*USER_TT_INPUT
  ○ CUMU_TT[COMP_1] = ADJ_TRANSIT_TIME[COMP_1]
  ○ CUMU_TT[COMP_2] = ADJ_TRANSIT_TIME[COMP_1]+ADJ_TRANSIT_TIME[COMP_2]
  ○ CUMU_TT[COMP_3] =
    ADJ_TRANSIT_TIME[COMP_1]+ADJ_TRANSIT_TIME[COMP_2]+ADJ_TRANSIT_TIME[COMP_
    3]
  ○ CUMU_TT[COMP_4] =
    ADJ_TRANSIT_TIME[COMP_1]+ADJ_TRANSIT_TIME[COMP_2]+ADJ_TRANSIT_TIME[COMP_
    3]+ADJ_TRANSIT_TIME[COMP_4]
  ○ CUMU_TT[COMP_5] =
    ADJ_TRANSIT_TIME[COMP_1]+ADJ_TRANSIT_TIME[COMP_2]+ADJ_TRANSIT_TIME[COMP_
    3]+ADJ_TRANSIT_TIME[COMP_4]+ADJ_TRANSIT_TIME[COMP_5]
  ○ TRANSFERS[COMP_1] = LOGN(10)/ADJ_TRANSIT_TIME[COMP_1]
  ○ TRANSFERS[COMP_2] = LOGN(10)/ADJ_TRANSIT_TIME[COMP_2]
  ○ TRANSFERS[COMP_3] = LOGN(10)/ADJ_TRANSIT_TIME[COMP_3]
  ○ TRANSFERS[COMP_4] = LOGN(10)/ADJ_TRANSIT_TIME[COMP_4]
  ○ TRANSFERS[COMP_5] = LOGN(10)/ADJ_TRANSIT_TIME[COMP_5]
  ○ USER_TT_INPUT = 1
```

All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

The invention now being fully described, it will be apparent to one of ordinary skill in the art that many changes and modifications can be made thereto without departing from the spirit or scope of the appended claims.

CLAIMS

What is claimed is:

1. A computer-implemented method of predicting a pharmacokinetic property of a target compound in an anatomical segment of a target mammalian system from a pharmacokinetic property of a test compound in an anatomical segment of a data source system, said computer comprising as operably linked components:

(a) an input/output system,

(b) a simulation engine, and

(c) a stored physiologic pharmacokinetic simulation model of said mammalian system, said simulation model comprising:

differential equations for calculating a change in one or more physiological parameters of said target mammalian system and the movement and disposition of said target compound in said mammalian system as a function of time, using input data for said differential equations comprising a pharmacokinetic property of the test compound in the anatomical segment of said data source system; and

a logic function module having control statement rules for initiating said physiologic pharmacokinetic simulation model of said mammalian system function,

wherein said model generates estimated values for a selected pharmacokinetic property of said target compound when supplied with input values corresponding to said selected pharmacokinetic property of said test compound in a portion of said data source system

said method comprising:

(a) entering into said input/output system input data comprising the pharmacokinetic property of said test compound in the segment of said data source system; and

(b) applying said simulation engine and said simulation model, and initiating said estimation function to predict said pharmacokinetic property of said target compound in a segment of said target mammalian system.

2. A computer-implemented method of predicting a pharmacokinetic property of a compound in a first anatomical segment of a mammalian system of interest from a pharmacokinetic property of said compound in a second anatomical segment of said mammalian system of interest, said method comprising:

providing a computer having as operably linked computer-implemented components an input/output system, a simulation engine, and a physiologic pharmacokinetic simulation model of at least first and second anatomical segments of said mammalian system of interest, said simulation model comprising (i) differential equations for calculating the change in one or more physiological parameters of said first and second segments and the movement and disposition of said compound in said first and second segments as a function of time, and (ii) a logic function module having a regional correlation parameter estimation function and a control statement for initiating said function, wherein said estimation function when initiated is capable of generating an estimated value for a selected pharmacokinetic property comprising an absorption parameter of said compound in said first segment when supplied with an input value corresponding to said selected pharmacokinetic property of said compound in said second segment and with a regional correlation coefficient for said selected pharmacokinetic parameter of said first and second segments;

entering into said input/output system input data comprising a pharmacokinetic property of said compound in said second segment; and

applying said simulation engine and said simulation model, and initiating said estimation function to predict said pharmacokinetic property of said compound in said first segment of said mammalian system of interest.

3. The method of claim 2, wherein said regional correlation estimation function comprises a function/transformation algorithm.

4. The method of claim 3, wherein said function/transformation algorithm is selected from the group consisting of a polynomial, exponential, and logarithm.

5. The method of claim 2, wherein said regional correlation coefficient comprises a best fit value that transforms said input data comprising said pharmacokinetic property of said compound in said second segment to an estimated pharmacokinetic property of said compound in said first segment.

6. The method of claim 2, wherein said pharmacokinetic property is selected from the group consisting of absorption, distribution, metabolism, elimination and toxicity.

7. The method of claim 2, wherein said pharmacokinetic parameter is selected from the group consisting of permeability, solubility, dissolution rate and transport mechanism.

8. The method of claim 2, wherein said differential equations are selected from the group consisting of equations for fluid transit, fluid absorption, mass transit, mass dissolution, mass solubility, and mass absorption.

9. The method of claim 2, which further comprises reversibly storing said estimated value for said pharmacokinetic parameter of said compound in said first segment in a computer-implemented database.

10. The method of claim 2, which further comprises reversibly storing in a computer-implemented database an output value corresponding to said pharmacokinetic property of said compound in a segment of said mammalian system that is generated by applying said simulation engine and said simulation model.

11. The method of claim 2, wherein said mammalian system of interest is selected from the group consisting of gastrointestinal tract, liver, heart, kidney, eye, nose, lung, skin and brain.

12. The method of claim 2, wherein said mammalian system of interest is human.

13. The method of claim 2, wherein said input data comprises in vitro data.

14. The method of claim 13, wherein said in vitro data is derived from testing of said compound in an assay that generates data selected from the group consisting of

cell, tissue, physicochemical, structure-activity relationship (SAR) SAR, and quantitative structure-activity relationship (QSAR) QSAR data.

15. The method of claim 2, wherein said computer is a computer system having a data processor, a memory and a display.

16. The method of claim 2, wherein said computer is a standalone computer having a data processor, a memory and a display.

17. The method of claim 2, wherein said computer-implemented components comprise computer readable program code.

18. The method of claim 17, wherein said computer readable program code is embodied in a computer readable medium.

19. A computer-implemented method of simulating one or more parameters of absorption of a compound in a mammalian system of interest using regional correlation parameter estimation, said method comprising:

providing a computer having as operably linked computer-implemented components an input/output system, a simulation engine, and a physiologic pharmacokinetic simulation model of at least two segments of mammalian system of interest having one or more absorption barriers to a compound based on the selected route of administration, said simulation model comprising (i) differential equations for calculating one or more parameters of absorption of said compound in said segments as a function of time and (ii) a logic function module having a regional correlation parameter estimation function and a control statement for initiating said estimation function, said estimation function when initiated being capable of generating an estimated value for a parameter of absorption of said compound in a first segment of said mammalian system utilizing an input value for said parameter of absorption of said compound in a second segment of said mammalian system;

entering through said input/output system input data comprising a parameter of absorption for said compound in said second segment; and applying said simulation engine and said simulation model, and initiating said estimation function to simulate one or more parameters of absorption of said compound in said first segment of said mammalian system of interest.

20. A method of simulating a pharmacokinetic parameter of a compound in a mammalian system of interest, said method comprising:

providing a computer having as operably linked computer-implemented components an input/output system, a simulation engine, and a physiologic pharmacokinetic simulation model of one or more segments of a selected mammalian system having one or more physiological barriers to absorption of said compound based on a selected route of administration, said simulation model comprising: (i) differential equations for one or more of fluid transit, fluid absorption, mass transit, mass dissolution, mass solubility, and mass absorption for one or more segments of said mammalian system; (ii) a regional correlation parameter estimation function for one or more of permeability, solubility, dissolution rate and transport mechanism; (iii) initial parameter values for said differential equations corresponding to physiological parameters and one or more regional correlation parameters for one or more segments of said mammalian system; and (iv) control statement rules for one or more of transit, absorption, permeability, solubility, dissolution, and concentration for one or more segments of said mammalian system;

entering through said input/output system input data comprising dose, permeability and solubility data for said compound for one or more segments of said mammalian system; and

applying said simulation engine and said simulation model to simulate one or more pharmacokinetic parameters of said compound relative to one or more segments of said mammalian system.

21. The method of claim 20, wherein said pharmacokinetic parameters of said compound relative to one or more segments of said mammalian system are selected from the group consisting of absorption, distribution, metabolism, elimination and toxicity.

22. A method of simulating absorption of a compound in a mammal utilizing a pharmacokinetic simulation tool (PK tool), said method comprising:

providing a computer-implemented PK tool comprising an input/output system, a simulation engine, and a simulation model of one or more segments of a mammalian system of interest having one or more physiological barriers to absorption based on the selected route of administration, said simulation model comprising as operably linked components: (i) differential equations for one or more of fluid transit, fluid absorption, mass transit, mass dissolution, mass solubility, and mass absorption for one or more segments of said mammal system; (ii) initial parameter values for said differential equations corresponding to physiological parameters and selectively optimized adjustment parameters, and optionally regional correlation parameters, for one or more segments of said mammal system; and (iii) control statement rules for one or more of transit, absorption, permeability, solubility, dissolution, and concentration for one or more segments of said mammal system;

entering into said input/output system input data comprising dose, permeability and solubility data for said compound for one or more of said segments of said mammal system; and

applying said simulation engine and said simulation model to simulate absorption of said compound in said mammal system.

23. A computer-implemented method of predicting a pharmacokinetic property of a compound in a mammalian system of interest, said method comprising:

providing a computer comprising as operably linked computer-implemented components an input/output system, a simulation engine, and a physiologic pharmacokinetic simulation model of two or more segments of a mammalian system of interest, wherein said simulation model comprises differential equations for calculating as a function of time the change in (i) a physiological parameter of one or more of said segments and (ii) a pharmacokinetic property comprising an absorption parameter of a compound relative to a selected route of administration, barrier to absorption and sampling site of one or more of said segments, and wherein one or more of said differential equations is modified by a selectively optimized adjustment parameter;

entering through said input/output system input data comprising dose, permeability and solubility data for said compound for one or more of said segments of said mammal system; and

applying said simulation engine and said simulation model to predict a pharmacokinetic property of said compound in one or more segments of said mammal system of interest.

24. The method of claim 23, wherein said computer is a computer system having a data processor, a memory and a display.

25. The method of claim 23 wherein said computer is a standalone computer having a data processor, a memory and a display.

26. The method of claim 23, wherein said computer-implemented components comprise computer readable program code.

27. The method of claim 26, wherein said computer readable program code is embodied in a computer readable medium.

28. The method of claim 26, wherein said computer readable program code is embodied in said memory.

29. The method of claim 23, wherein said input/output system comprises a user interface.

30. The method of claim 23, wherein said simulation engine comprises a differential equation solver.

31. The method of claim 23, wherein said differential equations are for fluid transit, fluid absorption, mass transit, mass dissolution, mass solubility, and mass absorption.

32. The method of claim 23, wherein said pharmacokinetic property is selected from the group consisting of absorption, distribution, metabolism, elimination and toxicity.

33. The method of claim 23, wherein said absorption parameter is selected from the group consisting of concentration, permeability, solubility, dissolution rate, transport mechanism, and formulation release rate.

34. The method of claim 23, wherein said physiological parameter is selected from the group consisting of pH, initial fluid volume, surface area, transit time, fluid volume transfer rate, and fluid absorption.

35. The method of claim 23, wherein said mammalian system of interest is human.

36. The method of claim 23, wherein said mammalian system of interest is selected from the group consisting of gastrointestinal tract, liver, heart, kidney, eye, nose, lung, skin and brain.

37. The method of claim 23, wherein said simulation model comprises one or more control statement rules.

38. The method of claim 37, wherein said control statement rules are for controlling simulation of one or more of transit, absorption, permeability, solubility, dissolution, and concentration for one or more segments of said mammal system of interest.

39. The method of claim 23, wherein said input data further comprises data selected from the group consisting of dissolution rate, transport mechanism and formulation release rate.

40. The method of claim 23, wherein said equations comprise one or more input variables corresponding to said input data for calculating as one or more output variables said change in said physiological parameter.

41. The method of claim 23, wherein said equations comprise one or more input variables corresponding to said input data for calculating as one or more output variables said change in said absorption parameter.

42. The method of claim 23, wherein said selectively optimized adjustment parameter correlates said input data to output data comprising said pharmacokinetic property of said compound.

43. The method of claim 42, wherein said input data comprises in vitro data and said selectively optimized adjustment parameter comprises a best fit value obtained by (i) assigning an initial value to a selected adjustment parameter of said simulation model, (ii) fitting a combination of in vitro data and in vivo data for different compounds of a compound test set with said simulation model, (iii) selecting a best fit value for selected adjustment parameter that, when assigned as an initial value to said selected adjustment parameter, permits said simulation model to predict said pharmacokinetic property of said compound when said input data comprises said in vitro data, and (iv) assigning said best fit value to said selected adjustment parameter so as to generate said selectively optimized adjustment parameter.

44. The method of claim 43, wherein said in vitro data is obtained from testing of said compound in one or more assays that generate data selected from the group consisting of cell, tissue, structure-activity relationship (SAR), and quantitative structure-activity relationship (QSAR) data.

45. The method of claim 43, wherein said different compounds of a compound test set comprise compounds having diverse pharmacokinetic properties.

46. The method of claim 42, wherein said input data comprises in vivo data from a first species of mammal and said mammalian system of interest corresponds to a second species of mammal, and wherein said selectively optimized adjustment parameter comprises a best fit value obtained by (i) assigning an initial value to a selected adjustment parameter of said simulation model, (ii) fitting a combination of in vivo data with said simulation model, said combination of in vivo data being derived from testing of different compounds of a compound test set in said first species of mammal and said second species of mammal, (iii) selecting a best fit value for selected adjustment parameter that, when assigned as an initial value to said selected adjustment parameter, permits said simulation model to predict said pharmacokinetic property of said compound when said input data comprises said in vitro data from said first species of mammal, and (iv) assigning said best fit value to said selected adjustment parameter so as to generate said selectively optimized adjustment parameter.

47. The method of claim 46, wherein said different compounds of a compound test set comprise compounds having diverse pharmacokinetic properties.

48. The method of claim 23, wherein said selectively optimized adjustment parameter is for one or more of fluid absorption, flux, permeability, transport mechanism, transfer rate, and segment surface area.

49. The method of claim 22 or 23, which further comprise reversibly storing in a computer-implemented database data corresponding to a predicted pharmacokinetic property of said compound.

50. The method of claim 23, wherein said physiologic pharmacokinetic simulation module comprises at least two of said anatomical segments and a logic function model comprising a regional correlation estimation function and a control statement for initiating said function, wherein said estimation function when initiated is capable of generating an estimated value for a selected pharmacokinetic property of said compound in a first anatomical segment when supplied with an input value corresponding to said selected pharmacokinetic property in a second anatomical segment and with a regional correlation coefficient for said selected pharmacokinetic property of said first and second anatomical segments.

51. The method of claim 50, wherein said regional correlation estimation function comprises a function/transformation algorithm.

52. The method of claim 51, wherein said function/transformation algorithm is selected from the group consisting of a polynomial, exponential, and logarithm.

53. The method of claim 50, wherein said regional correlation coefficient comprises a best fit value that transforms said input data comprising said pharmacokinetic property of said compound in said second segment to an estimated pharmacokinetic property of said compound in said first segment.

54. A method of selectively optimizing a simulation model for predicting pharmacokinetic property of a compound in a mammalian system of interest, said method comprising the steps of:

(i) generating selectively optimized adjustment parameter values for two or more independent parameters of said simulation model utilizing a curve-fitting algorithm that simultaneously estimates the change required in said parameter values necessary for predicting a pharmacokinetic property of said compound in said mammalian system of interest when said simulation model is supplied with one or more input variables corresponding to a pharmacokinetic property of a compound test set derived from (a) a first data source corresponding to the mammalian system of interest, and (b) a second data source corresponding to a system other than the mammalian system of interest;

(ii) selecting adjustment parameter values that permit correlation of one or more of the input variables from the first data source to one or more input variables from the second data source;

(iii) repeating steps (i) and (ii) one or more times for one or more additional independent parameters of said simulation model until deviation of predictability using said first data source as input data into said simulation model from predictability using said second data source as input into said simulation model is minimized; and

(iv) utilizing said selectively optimized adjustment parameters as constants for said independent parameters in said simulation model.

55. A computer-implemented method of generating a selectively optimized value for an adjustment parameter of a physiologic-based simulation model for predicting an in vivo property of a compound in a mammalian system of interest from an in vitro property of said compound, said method comprising:

(i) providing a computer having as operably linked computer-implemented components a curve-fitting algorithm and a physiologic-based simulation model of a mammalian system of interest, wherein said simulation model comprises one or more equations having an input variable for calculating as an output variable an in vivo property of a compound in said mammalian system as a function of time, and wherein one or more of said equations is modified by an adjustment parameter;

(ii) using said computer and said curve-fitting algorithm, fitting with said simulation model a combination of in vitro data and in vivo data for different compounds of a compound test set, wherein said in vitro data and said in vivo data correspond to one or more input variables of said equations, and optionally one or more output variables of said equations, and wherein said fitting generates one or more best fit values for said adjustment parameter; and

(iii) generating with said computer a selectively optimized value for said adjustment parameter of said simulation model by selecting one or more of said best fit values that, when assigned as an initial value to said adjustment parameter, permit said simulation model to predict an in vivo property of a compound in said mammalian system when in vitro data for said compound that corresponds to one or more input variables of said equations is utilized as input into said simulation model.

56. The method of claim 55, wherein said physiologic-based simulation model comprises a physiologic pharmacokinetic model of one or more anatomical segments of said mammalian system of interest.

57. The method of claim 56, wherein said physiologic pharmacokinetic model comprises differential equations for calculating the change in one or more physiological parameters of one or more of said anatomical segments and the movement and disposition of said compound in one or more of said anatomical segments as a function of time.

58. The method of claim 56, wherein said mammalian system of interest is selected from the group consisting of gastrointestinal tract, liver, heart, kidney, eye, nose, lung, skin and brain.

59. The method of claim 56, wherein said mammalian system of interest is human.

60. The method of claim 57, wherein one or more of said differential equations is for calculating a variable of a parameter corresponding to one or more in vivo properties of said compound selected from the group consisting of absorption, distribution, metabolism, elimination, and toxicity.

61. The method of claim 55, wherein said different compounds of said compound test set include compounds exhibiting a diverse range of in vivo properties in said mammalian system of interest.

62. The method of claim 61, wherein said in vivo properties are selected from the group consisting of permeability, solubility, dissolution, activity, metabolism, and toxicity.

63. The method of claim 55, wherein said in vivo data is derived from testing of a compound for an in vivo property in said mammalian system of interest.

64. The method of claim 55, wherein said in vitro data is derived from testing of a compound for an in vitro property in an assay that generates data selected from the group consisting of cell, tissue, physicochemical, structure-activity relationship (SAR) SAR, and quantitative structure-activity relationship (QSAR) QSAR data.

65. The method of claim 55, wherein said in vitro data and said in vivo data comprise a variable of a parameter corresponding to one or more in vitro and in vivo properties of said compound selected from the group consisting of absorption, distribution, metabolism, elimination, and toxicity.

66. The method of claim 55, wherein said fitting is simultaneous.

67. The method of claim 55, wherein said curve-fitting algorithm is a regression-based algorithm.

68. The method of claim 55, which further comprises reversibly storing said selectively optimized value for said adjustment parameter in a computer-implemented database.

69. The method of claim 55, which further comprises repeating steps (i) to (iii) one or more times for one or more additional adjustment parameters.

70. A computer-implemented method of generating a selectively optimized value for an adjustment parameter of a physiologic-based simulation model for predicting an in vivo property of a compound in a first mammalian system of interest from an in

vivo property of said compound in a second mammalian system of interest, said method comprising:

(i) providing a computer having as operably linked computer-implemented components a curve-fitting algorithm and a physiologic-based simulation model of a first mammalian system of interest, wherein said simulation model comprises one or more equations having an input variable for calculating as an output variable an in vivo property of a compound in said first mammalian system as a function of time, and wherein one or more of said equations is modified by an adjustment parameter;

(ii) using said computer and said curve-fitting algorithm, fitting with said simulation model a combination of in vivo data for different compounds of a compound test set derived from testing of said different compounds in said first mammalian system and in said second mammalian system, wherein said in vivo data corresponds to one or more input variables of said equations, and optionally one or more output variables of said equations, and wherein said fitting generates one or more best fit values for said adjustment parameter; and

(iii) generating with said computer a selectively optimized adjustment value for said adjustment parameter of said simulation model by selecting one or more of said best fit values that, when assigned as an initial value to said adjustment parameter, permit said simulation model to predict an in vivo property of a compound in said first mammalian system when in vivo data for said compound that is derived from testing of said compound in said second mammalian system and that corresponds to one or more input variables of said equations is utilized as input into said simulation model.

71. A computer-implemented method of selectively optimizing a physiologic-based simulation model for predicting an in vivo property of a compound in a mammalian system of interest from an in vitro property of said compound, said method comprising:

(i) providing a computer having as operably linked computer-implemented components a curve-fitting algorithm and a physiologic-based simulation model of a mammalian system of interest, said simulation model having equations for independent parameters comprising physiological parameters of said mammalian system and physicochemical parameters of a compound in said mammalian system, said equations having input variables for calculating as an output variable an in vivo property of a compound in said mammalian system as a function of time, and wherein one or more of said independent parameters is modified by an adjustment parameter;

(ii) using said computer and said curve-fitting algorithm, fitting with said simulation model a combination of in vitro data and in vivo data for different compounds of a compound test set, wherein said in vitro data and said in vivo data correspond to one or more input variables of said equations, and optionally one or more output variables of said equations, and wherein said fitting generates one or more best fit values for said adjustment parameter; and

(iii) generating with said computer a selectively optimized value for said adjustment parameter for one or more independent parameters of said simulation model by selecting one or more of said best fit values that, when assigned as an initial value to said adjustment parameter, permit said simulation model to predict an in vivo property of a compound in said mammalian system when in vitro data for said compound that corresponds to one or more input variables of said equations is utilized as input into said simulation model;

(iv) repeating steps (i) through (iii) one or more times for one or more additional independent parameters of said simulation model until deviation of predictability of said in vivo property of said compound in said mammalian system is minimized; and

(v) utilizing said selectively optimized values for said adjustment parameters as constants for said independent parameters in said simulation model for predicting said in vivo property of a compound in said mammalian system of interest.

72. A computer-implemented method for selectively optimizing a physiologic pharmacokinetic simulation model for predicting a pharmacokinetic property of a compound in a mammalian system of interest, said method comprising the steps of:

(i) providing a computer having as operably linked computer-implemented components a curve-fitting algorithm and a physiologic pharmacokinetic simulation model of a mammalian system of interest, wherein said simulation model comprises differential equations having input variables for calculating as an output variable a pharmacokinetic property of a compound in said mammalian system as a function of time, and wherein said equations include one or more independent parameters modified by an adjustment parameter,

(ii) using said computer and said curve-fitting algorithm, generating selectively optimized values for said adjustment parameter for one or more of said independent parameters by fitting with said simulation model a combination of data for different compounds of a compound test set, wherein said data correspond to one or more of said input variables, and optionally one or more of said output variables, and wherein said data is derived from (a) a first data source corresponding to the mammalian system of interest, and (b) a second data source corresponding to a system other than the mammalian system of interest;

(iii) selecting one or more best fit values for said adjustment parameter that, when assigned as an initial value for said adjustment parameter, permit correlation of one or more of said input variables from said first data source to one or more of said input variables from said second data source when using either or both of said first data source and said second data source as input variables in said simulation model to predict said pharmacokinetic property;

(iv) repeating steps (i) and (ii) one or more times for one or more additional independent parameters of said simulation model until deviation of predictability of said pharmacokinetic property when using either or both of said first data source and said second data source as input variables in said simulation model is minimized; and

(v) utilizing said selectively optimized values as constants for said adjustment parameters in said simulation model for predicting said pharmacokinetic property of a compound in said mammalian system of interest.

73. A computer-implemented method of generating a selectively optimized adjustment parameter of a physiologic pharmacokinetic simulation model of a mammalian system of interest for predicting a pharmacokinetic property of a compound in said mammalian system from in vitro data, said method comprising:

(i) providing a computer having as a computer implemented physiologic pharmacokinetic simulation model of a mammalian system of interest, said simulation model comprising equations having one or more input variables for calculating as one or more output variables a pharmacokinetic property of a compound in said mammalian system as a function of time, wherein one or more of said equations is modified by an adjustment parameter;

(ii) assigning an initial value to a selected adjustment parameter of said simulation model;

(iii) fitting a combination of in vitro data and in vivo data for different compounds of a compound test set with said simulation model utilizing a curve fitting algorithm that estimates the change required in said initial value in order to change one or more of said output variables;

(iv) selecting a best fit value for said selected adjustment parameter that, when assigned as an initial value to said selected adjustment parameter, permits said simulation model to predict said pharmacokinetic property of said compound when said input data comprises said in vitro data;

(v) assigning said best fit value as a constant to said selected adjustment parameter so as to generate said selectively optimized adjustment parameter that modifies one or more of said equations; and

(vi) optionally repeating steps (i) through (v) one or more times for one or more additional adjustment parameters.

74. The method of claim 73, wherein said in vitro data is obtained from testing of a compound in one or more assays that generate data selected from the group consisting of cell, tissue, structure-activity relationship (SAR), and quantitative structure-activity relationship (QSAR) data.

75. The method of claim 73, wherein said different compounds of a compound test set comprise compounds having diverse pharmacokinetic properties in said mammalian system of interest.

76. A computer-implemented method of generating a selectively optimized adjustment parameter of a physiologic pharmacokinetic simulation model of a mammalian system of interest that corresponds to a second species of mammal for

predicting a pharmacokinetic property of a compound in said mammalian system from in vivo data obtained from a first species of mammal, said method comprising:

(i) providing a computer having as a computer implemented physiologic pharmacokinetic simulation model of a mammalian system of interest, said simulation model comprising equations having one or more input variables for calculating as one or more output variables a pharmacokinetic property of a compound in said mammalian system as a function of time, wherein one or more of said equations is modified by an adjustment parameter;

(ii) assigning an initial value to a selected adjustment parameter of said simulation model;

(iii) fitting a combination of in vivo data with said simulation model, said combination of in vivo data derived from testing of different compounds of a compound test set in said first species of mammal and said second species of mammal, and said fitting is performed utilizing a curve fitting algorithm that estimates the change required in said initial value in order to change one or more of said input variables, and optionally one or more of said output variables;

(iv) selecting a best fit value for said selected adjustment parameter that, when assigned as an initial value to said selected adjustment parameter, permits said simulation model to predict said pharmacokinetic property of said compound when said input data comprises said in vivo data from said first species of mammal;

(v) assigning said best fit value as a constant to said selected adjustment parameter so as to generate said selectively optimized adjustment parameter that modifies one or more of said equations; and

(vi) optionally repeating steps (i) through (v) one or more times for one or more additional adjustment parameters.

77. The method of claim 76, wherein said different compounds of a compound test set comprise compounds having diverse pharmacokinetic properties in said mammalian system of interest.

78. The method of claim 73 or 76, wherein said selectively optimized adjustment parameter is for one or more of fluid absorption, flux, permeability, transport mechanism, transfer rate, dissolution, solubility and segment surface area.

79. The method of claim 73 or 76, which further comprises reversibly storing said constant for said selectively optimized adjustment parameter in a computer-implemented database.

80. A computer system configured to predict a pharmacokinetic property of a target compound in a target anatomical segment of a target mammalian system from a pharmacokinetic property of a test compound in an anatomical segment of a data source system, said computer comprising as operably linked components:

(a) an input/output system,

(b) a simulation engine, and

(c) a stored physiologic pharmacokinetic simulation model of said target mammalian system, said simulation model comprising:

(i) differential equations for calculating a change in one or more physiological parameters of said target mammalian system and the movement and disposition of said target compound in said target mammalian system as a function of time, using input data for said differential equations comprising the pharmacokinetic property of the test compound in the anatomical segment of said data source system; and

(ii) boundary condition parameter values for said differential equations corresponding to parameters of said target mammalian system, and

(iii) a logic function module having control statement rules for initiating said physiologic pharmacokinetic simulation model of said mammalian system function.

81. The computer system of claim 80 wherein said computer, using the model, generates estimated values for a selected pharmacokinetic property of said target compound when supplied with input values corresponding to said selected pharmacokinetic property of said test compound in a portion of said data source system by the method comprising:

(a) entering into said input/output system input data comprising a pharmacokinetic property of said test compound in a segment of said data source system; and

(b) applying said simulation engine and said simulation model, and invoking said simulation engine model for predicting said pharmacokinetic property of said target compound in a segment of said target mammalian system.

82. A computer system for simulating absorption of a compound in a mammal, said system having as computer-implemented components an input/output system, simulation engine, and simulation model of one or more segments of a selected mammalian system having one or more physiological barriers to absorption based on a selected route of administration, said simulation model comprising as operably linked components:

(i) differential equations for one or more of fluid transit, fluid absorption, mass transit, mass dissolution, mass solubility, and mass absorption for one or more segments of said mammalian system; (ii) initial parameter values for said differential equations corresponding to physiological parameters and selectively optimized adjustment parameters, and optionally regional correlation parameters, for one or more segments of said mammalian system; and (iii) control statement rules for one or more of transit, absorption, permeability, solubility, dissolution, and concentration for one or more segments of said mammalian system;

said input/output system, said simulation engine, and said simulation model being capable of working together to carry out the steps of:

(a) receiving as input data through said input/output system, dose, permeability and solubility data for said compound for one or more segments of said mammalian system; and

(b) applying said simulation engine and said simulation model to simulate absorption of said compound relative to one or more segments of said mammalian system.

83. A computer system for simulating a pharmacokinetic property of a compound in a mammalian system of interest, said computer system comprising as operably linked computer-implemented components an input/output system, a simulation engine, and a physiologic pharmacokinetic simulation model of one or more anatomical segments of said mammalian system of interest, said simulation model comprising differential equations for calculating as a function of time the change in (i) a physiological parameter of one or more of said segments and (ii) a pharmacokinetic property comprising an absorption parameter of a compound relative to a selected route of administration, barrier to absorption and sampling site of one or more of said segments, wherein one or more of said differential equations is modified by a selectively optimized adjustment parameter;

said input/output system, said simulation engine, and said physiologic pharmacokinetic simulation model being capable of working together to carry out the steps of:

(a) receiving through said computer readable input/output system input data comprising dose, permeability and solubility data for said compound for one or more segments of said mammalian system of interest; and

(b) applying said simulation engine and said physiologic pharmacokinetic simulation model to simulate a pharmacokinetic property of said compound relative to one or more segments of said mammalian system of interest.

84. The computer system of claim 83, wherein said differential equations are for one or more of fluid transit, fluid absorption, mass transit, mass dissolution, mass solubility, and mass absorption for one or more segments of said mammalian system.

85. The computer system of claim 83, wherein said differential equations comprise initial parameter values corresponding to said physiological parameter and said selectively optimized adjustment parameter for one or more segments of said mammalian system.

86. The computer system of claim 83, wherein said physiologic pharmacokinetic simulation model comprises control statement rules for one or more of transit,

absorption, permeability, solubility, dissolution, and concentration for one or more segments of said mammalian system.

87. The computer system of claim 86, wherein said control statement rules are IF...THEN production rules.

88. The computer system of claim 83, wherein said input/output system comprises a user interface.

89. The computer system of claim 83, wherein said simulation engine comprises a differential equation solver.

90. The computer system of claim 83, wherein said pharmacokinetic property is selected from the group consisting of absorption, distribution, metabolism, elimination and toxicity.

91. The computer system of claim 83, wherein said absorption parameter is selected from the group consisting of concentration, permeability, solubility, dissolution rate, transport mechanism, and formulation release rate.

92. The article of manufacture of claim 83, wherein said physiological parameter is selected from the group consisting of pH, fluid volume, fluid volume transfer rate, fluid absorption, surface area, and transit time.

93. The computer system of claim 83, wherein said mammalian system of interest is selected from the group consisting of gastrointestinal tract, liver, heart, kidney, eye, nose, lung, skin and brain.

94. The computer system of claim 93, wherein said mammalian system of interest is gastrointestinal tract and said segments are selected from the group consisting of stomach, duodenum, jejunum, ileum and colon.

95. The computer system of claim 83, wherein said input data includes data selected from the group consisting of dissolution rate, transport mechanism and formulation release rate.

96. The computer system of claim 83, wherein said differential equations comprise one or more input variables corresponding to said input data for calculating as one or more output variables said change in said physiological parameter.

97. The computer system of claim 83, wherein said differential equations comprise one or more input variables corresponding to said input data for calculating as one or more output variables said change in said absorption parameter.

98. The computer system of claim 83, wherein said selectively optimized adjustment parameter correlates said input data to output data comprising said pharmacokinetic property of said compound.

99. The computer system of claim 98, wherein said input data comprises in vitro data and said selectively optimized adjustment parameter comprises a best fit value obtained by (i) assigning an initial value to a selected adjustment parameter of said simulation model, (ii) fitting a combination of in vitro data and in vivo data for different compounds of a compound test set with said simulation model, (iii) selecting a best fit value for said selected adjustment parameter that, when assigned as an initial value to said selected adjustment parameter, permits said simulation model to predict said pharmacokinetic property of said compound when said input data comprises said in vitro data, and (iv) assigning said best fit value as a constant to said selected adjustment parameter so as to generate said selectively optimized adjustment parameter that modifies one or more of said differential equations.

100. The computer system of claim 99, wherein said in vitro data is obtained from testing of a compound in one or more assays that generate data selected from the group consisting of cell, tissue, structure-activity relationship (SAR), and quantitative structure-activity relationship (QSAR) data.

101. The computer system of claim 99, wherein said different compounds of a compound test set comprise compounds having diverse pharmacokinetic properties in said mammalian system of interest.

102. The computer system of claim 98, wherein said input data comprises in vivo data from a first species of mammal and said mammalian system of interest comprises a second species of mammal, and wherein said selectively optimized adjustment

parameter comprises a best fit value obtained by (i) assigning an initial value to a selected adjustment parameter of said simulation model, (ii) fitting a combination of in vivo data with said simulation model, said combination of in vivo data derived from testing of different compounds of a compound test set in said first species of mammal and said second species of mammal, (iii) selecting a best fit value for said selected adjustment parameter that, when assigned as an initial value to said selected adjustment parameter, permits said simulation model to predict said pharmacokinetic property of said compound when said input data comprises said in vitro data from said first species of mammal, and (iv) assigning said best fit value as a constant to said selected adjustment parameter so as to generate said selectively optimized adjustment parameter that modifies one or more of said differential equations.

103. The computer system of claim 102, wherein said different compounds of a compound test set comprise compounds having diverse pharmacokinetic properties in said mammalian system of interest.

104. The computer system of claim 83, wherein said selectively optimized adjustment parameter is for one or more of fluid absorption, flux, permeability, transport mechanism, transfer rate, and segment surface area.

105. The computer system of claim 103, wherein said physiologic pharmacokinetic simulation model comprises at least two of said anatomical segments and a logic function module comprising a regional correlation estimation function and a control statement for initiating said function, wherein said estimation function when initiated is capable of generating an estimated value for a selected pharmacokinetic property of said compound in a first anatomical segment when supplied with an input value corresponding to said selected pharmacokinetic property in a second anatomical segment and with a regional correlation coefficient for said selected pharmacokinetic property of said first and second anatomical segments.

106. A computer system for simulating a pharmacokinetic property of a compound in a mammal of interest utilizing regional correlation parameter estimation, said computer system comprising as operably linked computer-implemented components an input/output system, a simulation engine, and a physiologic pharmacokinetic simulation model of at least two segments of a selected mammalian system of interest,

said physiologic pharmacokinetic simulation model comprising (i) differential equations for calculating the change in one or more physiological parameters of first and second segments of said mammalian system of interest and the movement and disposition of said compound in said first and second segments as a function of time, and (ii) a logic function module having a regional correlation parameter estimation function and a control statement for initiating said function, wherein said estimation function when initiated is capable of generating an estimated value for a selected pharmacokinetic property comprising an absorption parameter of said compound in said first segment when supplied with an input value corresponding to said selected pharmacokinetic property of said compound in said second segment and with a regional correlation coefficient for said selected pharmacokinetic parameter of said first and second segments;

said input/output system, said simulation engine, and said physiologic pharmacokinetic simulation model being capable of working together to carry out the steps of:

(a) receiving through said input/output system input data comprising a pharmacokinetic property of said compound in said second segment of said mammalian system of interest; and

(b) applying said simulation engine and said physiologic pharmacokinetic simulation model to initiate said estimation function to estimate said pharmacokinetic property of said compound in said first segment of said mammalian system of interest.

107. The computer system of claim 106, wherein said regional correlation estimation function comprises a function/transformation algorithm.

108. The computer system of claim 107, wherein said function/transformation algorithm is selected from the group consisting of a polynomial, exponential, and logarithm.

109. The computer system of claim 106, wherein said regional correlation coefficient comprises a best fit value that transforms said input data comprising said pharmacokinetic property of said compound in said second segment to an estimated pharmacokinetic property of said compound in said first segment.

110. The computer system of claim 106, wherein said pharmacokinetic property is selected from the group consisting of absorption, distribution, metabolism, elimination and toxicity.

111. The computer system of claim 106, wherein said pharmacokinetic parameter is selected from the group consisting of permeability, solubility, dissolution rate and transport mechanism.

112. The computer system of claim 106, wherein said differential equations are selected from the group consisting of equations for fluid transit, fluid absorption, mass transit, mass dissolution, mass solubility, and mass absorption.

113. The computer system of claim 106, wherein said mammalian system of interest is selected from the group consisting of gastrointestinal tract, liver, heart, kidney, eye, nose, lung, skin and brain.

114. The computer system of claim 83 or 106, wherein said mammalian system of interest is human.

115. The computer system of claim 106, wherein said input data comprises in vitro data.

116. The computer system of claim 115, wherein said in vitro data is derived from testing of said compound in an assay that generates data selected from the group consisting of cell, tissue, physicochemical, structure-activity relationship (SAR) SAR, and quantitative structure-activity relationship (QSAR) QSAR data.

117. The computer system of claim 106, wherein said computer system comprises a data processor, a memory and a display.

118. The computer system of claim 106, wherein said input/output system comprises a user interface.

119. The computer system of claim 106, wherein said simulation engine comprises a differential equation solver.

120. The computer system of claim 106, wherein said physiologic pharmacokinetic simulation model comprises a subsystem of said computer system.

121. The computer system of claim 106, wherein one or more of said differential equations is modified by a selectively optimized adjustment parameter.

122. A subsystem for use in a computer system for simulating oral absorption of a compound in a mammal, said subsystem comprising:

(i) a computer-implemented simulation model of one or more segments of the gastrointestinal (GI) track of a mammal comprising differential equations for one or more of fluid transit, fluid absorption, mass transit, mass dissolution, mass solubility, and mass absorption for one or more segments of the GI tract of said mammal; and

(ii) a computer-implemented database comprising initial parameter values for said differential equations corresponding to physiological parameters and selectively optimized adjustment parameters, and optionally regional correlation parameters, for one or more segments of the GI tract of said mammal.

123. A computer-implemented database according to claim 122 having a compartment-flow model data structure.

124. A subsystem for use in a computer system for simulating oral absorption of a compound in a mammal, said subsystem comprising:

(i) a computer-implemented simulation model of one or more segments of the gastrointestinal (GI) track of a mammal comprising differential equations for one or more of fluid transit, fluid absorption, mass transit, mass dissolution, mass solubility, and mass absorption for one or more segments of the GI tract of said mammal; and

(ii) a computer-implemented database comprising initial parameter values for said differential equations corresponding to physiological parameters and regional correlation parameters for one or more segments of the GI tract of said mammal.

125. A computer-implemented database according to claim 124 having a compartment-flow model data structure.

126. The subsystem of claim 122 or 124, wherein said computer-implemented database comprises computer-implemented control statement rules for one or more of

transit, absorption, permeability, solubility, dissolution, and concentration for one or more segments of the GI tract of said mammal.

127. A computer-implemented database for use in a computer system for simulating absorption of a compound in a mammal, said computer-implemented database comprising:

a computer-implemented physiologic-based simulation model of one or more segments of selected mammalian system of interest comprising (i) differential equations for one or more of fluid transit time, fluid absorption, mass transit time, mass dissolution, mass solubility, and mass absorption for one or more segments of said mammalian system; (ii) initial parameter values for said differential equations corresponding to physiological parameters and adjustment parameters, and optionally one or more regional correlation parameters, for one or more segments of said mammalian system; and (iii) control statement rules for one or more of transit time, absorption, permeability, dissolution, concentration, and mathematical error correction for one or more segments of said mammalian system;

wherein said computer-implemented physiologic-based simulation model comprises a compartment-flow data structure for calculating time-dependent rate of absorption, extent of absorption, and concentration of a compound at a sampling site across a physiological barrier of one or more segments of said mammalian system when applied in a simulation engine having a differential equation solver and a control statement module.

128. The computer-implemented database of claim 127, wherein said adjustment parameters are selected from the group consisting of regional fluid absorption, permeability, flux, active transport, carrier mediated transport, compound efflux, transfer rate, and surface area.

129. The computer-implemented database of claim 127, wherein said physiological parameters are selected from the group consisting of soluble mass transfer rate constant, permeability, solubility, dissolution rate, transport mechanism, pH, initial volume, surface area, transit time, fluid volume transfer rate, and fluid absorption rate.

130. The computer-implemented database of claim 127, wherein said regional correlation parameters are for permeability.

131. A computer-implemented physiological simulation model of the gastrointestinal (GI) track of a mammal for simulating oral absorption of a compound in said mammal, said physiological simulation model corresponding to a compartment-flow model comprising:

compartments characterized by fluid volume, fluid absorption, insoluble mass, soluble mass, and mass absorption for one or more of segments of the GI track of a mammal, wherein said compartments are operably linked through flow regulators and converters, wherein said flow regulators regulate flow among compartments and said converters modify said flow regulators, and wherein said flow regulators are characterized by fluid absorption rate, fluid transit rate, insoluble mass transit rate, insoluble mass dissolution rate, soluble mass transit rate, and soluble mass absorption rate.

132. The computer-implemented physiological simulation model of claim 131, wherein said converters are characterized by fluid volume, fluid volume absorption rate constant, fluid volume transit rate constant, insoluble mass, insoluble mass transit rate constant, dissolution rate constant, soluble mass, soluble mass transit rate constant, surface area, dissolved mass concentration and permeability.

133. The computer-implemented physiological simulation model of claim 131, which further comprises compartments characterized by formulation and flow regulators characterized by formulation transit rate and formulation release rate.

134. A computer-implemented gastrointestinal (GI) transit simulation model for simulating mass and fluid loss in the GI track of a mammal, said GI transit simulation model corresponding to a compartment-flow model comprising:

compartments characterized by fluid volume and fluid volume absorption for stomach, duodenum, jejunum, ileum and colon that are operably linked through flow regulators and one or more converters that modify one or more of said flow regulators, wherein said flow regulators are characterized by fluid volume absorption rate and fluid volume transit rate, and wherein said converters are characterized by selectively optimized adjustment parameter values for one or more of fluid absorption rate constant and fluid volume transit rate constant.

135. A computer-implemented solubility simulation model for simulating pH dependent solubility and dissolution of a compound in the gastrointestinal (GI) track of a mammal, said solubility simulation model corresponding to a compartment-flow model comprising:

compartments characterized by insoluble mass and soluble mass for stomach, duodenum, jejunum, ileum and colon that are operably linked through flow regulators and one or more converters that modify one or more of said flow regulators, wherein said flow regulators are characterized by insoluble mass transit rate, insoluble mass dissolution rate, and soluble mass transit rate, wherein said converters are characterized by insoluble mass, insoluble mass transit rate constant, insoluble mass dissolution rate constant, soluble mass, and soluble mass transit rate constant, and wherein one or more of said converters are characterized by selectively optimized adjustment parameters.

136. A computer-implemented absorption simulation model for simulating absorption of a compound in the gastrointestinal (GI) track of a mammal to at least the portal vein, said absorption simulation model corresponding to a compartment-flow model comprising:

compartments characterized by soluble mass and soluble mass absorption for stomach, duodenum, jejunum, ileum and colon that are operably linked through flow regulators and one or more converters that modify said flow regulators, where said flow regulators are characterized by insoluble mass transit rate, insoluble mass dissolution rate, soluble mass transit rate, soluble mass absorption rate, where said converters are characterized by insoluble mass, insoluble mass dissolution rate constant, soluble mass transit rate constant, surface area, dissolved mass concentration, and permeability, and wherein one or more of said converters are characterized by selectively optimized adjustment parameters.

137. An article of manufacture comprising a computer readable medium having computer readable program code embodied therein for simulating absorption of a target compound in a mammal of interest, and having

(a) computer readable simulation engine program code, and

(b) computer readable simulation model code of one or more segments of a selected mammalian system having one or more physiological barriers to absorption of said target compound, said computer readable simulation model comprising as operably linked components:

(i) differential equations for one or more of fluid transit, fluid absorption, mass transit, mass dissolution, mass solubility, and mass absorption for one or more segments of said mammalian system, and

(ii) control statement rules for one or more segments of said mammalian system;

said computer readable simulation engine code and simulation model code being being configured to carry out the steps of:

(a) receiving input data for a compound for one or more segments of a data source system; and

(b) applying said computer readable simulation engine code and said computer readable simulation model code to simulate absorption of said target compound relative to one or more segments of said target mammalian system.

138. An article of manufacture comprising a computer readable medium having computer readable program code embodied therein for simulating absorption of a compound in a mammal of interest, and having computer readable input/output system, computer readable simulation engine, and computer readable simulation model of one or more segments of a selected mammalian system having one or more physiological barriers to absorption of said compound based on a selected route of administration, said computer readable simulation model comprising as operably linked components:

(i) differential equations for one or more of fluid transit, fluid absorption, mass transit, mass dissolution, mass solubility, and mass absorption for one or more segments of said mammalian system; (ii) initial parameter values for said differential equations corresponding to physiological parameters and selectively optimized adjustment parameters, and optionally one or more regional correlation parameters, for one or more segments of said mammalian system; and optionally (iii) control statement rules for one or more of transit, absorption, permeability, solubility, dissolution, and concentration for one or more segments of said mammalian system;

said computer readable input/output system, said computer readable simulation engine, and said computer readable simulation model being capable of working together to carry out the steps of:

(a) receiving as input data through said computer readable input/output system, dose, permeability and solubility data for said compound for one or more segments of said mammalian system; and

(b) applying said computer readable simulation engine and said computer readable simulation model to simulate absorption of said compound relative to one or more segments of said mammalian system of interest.

139. The article of manufacture of claim138, wherein said mammalian system is selected from the group consisting of the gastrointestinal tract, the eye, the nose, the lung, the skin, and the brain.

140. The article of manufacture of claim 139, wherein said mammalian system is the gastrointestinal tract.

141. The article of manufacture of claim 140, wherein said segments are selected from the group consisting of stomach, duodenum, jejunum, ileum, and colon.

142. The article of manufacture of claim 138, wherein said simulation model corresponds to a compartment-flow model comprising compartments that are operably linked through flow regulators modified by one or more converters.

143. The article of manufacture of claim 142, wherein said compartments comprise one or more compartments characterized by a parameter selected from the group consisting of fluid volume, fluid absorption, formulation, insoluble mass, soluble mass, and soluble mass absorption.

144. The article of manufacture of claim 142, wherein said flow regulators are characterized by a parameter selected from the group consisting of fluid absorption

rate, fluid transit rate, formulation transit rate, formulation release rate, insoluble mass transit rate, insoluble mass dissolution rate, soluble mass transit rate, and soluble mass absorption rate.

145. The article of manufacture of claim 142, wherein said converters are characterized by a parameter selected from the group consisting of fluid volume, fluid volume absorption rate constant, fluid volume transit rate constant, insoluble mass, insoluble mass transit rate constant, dissolution rate constant, soluble mass, soluble mass transit rate constant, surface area, dissolved mass concentration and permeability.

146. The article of manufacture of claim 142, wherein one or more of said converters are characterized by a selectively optimized adjustment parameter.

147. The article of manufacture of claim 142, wherein one or more of said converters are characterized by a regional correlation parameter.

148. The article of manufacture of claim 138, wherein said control statement rules are IF...THEN production rules.

149. The article of manufacture of claim 138, wherein said physiological parameters are characterized by a parameter selected from the group consisting of soluble mass transfer rate constant, permeability, solubility, dissolution rate, and transport mechanism.

150. The article of manufacture of claim 138, wherein said physiological parameters are characterized by a parameter selected from the group consisting of pH, initial fluid volume, surface area, fluid volume transit time, insoluble mass transit time, soluble mass transit time, fluid volume transfer rate, and fluid absorption rate.

151. The article of manufacture of claim 138, wherein said mammal is human.

152. The article of manufacture of claim 138, where said input data further comprises data for said compound of interest selected from the group consisting of dissolution rate, transport mechanism, transit time, pH and formulation release rate.

153. The article of manufacture of claim 138, wherein said input data is *in vitro* data.

154. The article of manufacture of claim 153, wherein said *in vitro* data is permeability data derived from an assay selected from the group consisting of a cell-based assay and a tissue-based assay.

155. The article of manufacture of claim 153, wherein said *in vitro* data is transport mechanism data derived from an assay selected from the group consisting of a cell-based assay and a tissue-based assay.

156. The article of manufacture of claim 153, wherein said *in vitro* data is permeability data derived from structure-activity relationship data of said compound.

157. The article of manufacture of claim 153, wherein said *in vitro* data is dissolution rate data derived from structure-activity relationship data of said compound.

158. The article of manufacture of claim 153, wherein said *in vitro* data is solubility data derived from structure-activity relationship data of said compound.

159. A computer program product for simulating absorption of a compound in a mammal, and having computer readable program code input/output system, computer readable program code simulation engine, and computer readable program code simulation model of one or more segments of a selected mammalian system having one or more physiological barriers to absorption based on a selected route of administration, said computer readable program code simulation model comprising as operably linked components:

(i) differential equations for one or more of fluid transit, fluid absorption, mass transit, mass dissolution, mass solubility, and mass absorption for one or more segments of said mammalian system; (ii) initial parameter values for said differential equations corresponding to physiological parameters and selectively optimized adjustment parameters, and optionally regional correlation parameters, for one or more segments of said mammalian system; and (iii) control statement rules for one or more of transit, absorption, permeability, solubility, dissolution, and concentration for one or more segments of said mammalian system;

wherein said computer readable program code input/output system, said computer readable program code simulation engine, and said computer readable program code simulation model being capable of working together to carry out the steps of:

(a) receiving as input data through said computer readable program code input/output system, dose, permeability and solubility data for said compound for one or more segments of said mammalian system; and

(b) applying said computer readable program code simulation engine and said computer readable program code simulation model to simulate absorption of said compound relative to one or more segments of said mammalian system.

160. An article of manufacture comprising a computer readable medium having computer readable program code embodied therein for simulating a pharmacokinetic property of a compound in a mammal of interest, and having computer readable input/output system, computer readable simulation engine, and computer readable physiologic pharmacokinetic simulation model of one or more anatomical segments of a selected mammalian system, said computer readable physiologic pharmacokinetic simulation model comprising differential equations for calculating as a function of time the change in (i) a physiological parameter of one or more of said segments and (ii) a pharmacokinetic property comprising an absorption parameter of a compound relative to a selected route of administration, barrier to absorption and sampling site of one or more of said segments, wherein one or more of said differential equations is modified by a selectively optimized adjustment parameter;

said computer readable input/output system, said computer readable simulation engine, and said computer readable physiologic pharmacokinetic simulation model being capable of working together to carry out the steps of:

(a) receiving through said computer readable input/output system input data comprising dose, permeability and solubility data for said compound for one or more segments of said mammalian system of interest; and

(b) applying said computer readable simulation engine and said computer readable physiologic pharmacokinetic simulation model to simulate a pharmacokinetic property of said compound relative to one or more segments of said mammalian system of interest.

161. The article of manufacture of claim 160, wherein said differential equations are for one or more of fluid transit, fluid absorption, mass transit, mass dissolution, mass solubility, and mass absorption for one or more segments of said mammalian system.

162. The article of manufacture of claim 160, wherein said differential equations comprise initial parameter values corresponding to said physiological parameter and said selectively optimized adjustment parameter for one or more segments of said mammalian system.

163. The article of manufacture of claim 160, wherein said physiologic pharmacokinetic simulation model comprises control statement rules for one or more of transit, absorption, permeability, solubility, dissolution, and concentration for one or more segments of said mammalian system.

164. The article of manufacture of claim 163, wherein said control statement rules are IF...THEN production rules.

165. The article of manufacture of claim 160, wherein said input/output system comprises a user interface.

166. The article of manufacture of claim 160, wherein said simulation engine comprises a differential equation solver.

167. The article of manufacture of claim 160, wherein said absorption parameter is selected from the group consisting of concentration, permeability, solubility, dissolution rate, transport mechanism, and formulation release rate.

168. The article of manufacture of claim 160, wherein said physiological parameter is selected from the group consisting of pH, fluid volume, fluid volume transfer rate, fluid absorption, surface area, and transit time.

169. The article of manufacture of claim 160, wherein said mammalian system of interest is selected from the group consisting of gastrointestinal tract, liver, heart, kidney, eye, nose, lung, skin and brain.

170. The article of manufacture of claim 169, wherein said mammalian system of interest is gastrointestinal tract and said segments are selected from the group consisting of stomach, duodenum, jejunum, ileum and colon.

171. The article of manufacture of claim 160, wherein said input data includes data selected from the group consisting of dissolution rate, transport mechanism and formulation release rate.

172. The article of manufacture of claim 160, wherein said differential equations comprise one or more input variables corresponding to said input data for calculating as one or more output variables said change in said physiological parameter.

173. The article of manufacture of claim 160, wherein said differential equations comprise one or more input variables corresponding to said input data for calculating as one or more output variables said change in said absorption parameter.

174. The article of manufacture of claim 160, wherein said selectively optimized adjustment parameter correlates said input data to output data comprising said pharmacokinetic property of said compound.

175. The article of manufacture of claim 174, wherein said input data comprises *in vitro* data and said selectively optimized adjustment parameter comprises a best fit value obtained by (i) assigning an initial value to a selected adjustment parameter of said simulation model, (ii) fitting a combination of *in vitro* data and *in vivo* data for different compounds of a compound test set with said simulation model, (iii) selecting a best fit value for said selected adjustment parameter that, when assigned as an initial value to said selected adjustment parameter, permits said simulation model to predict said pharmacokinetic property of said compound when said input data comprises said *in vitro* data, and (iv) assigning said best fit value as a constant to said selected adjustment parameter so as to generate said selectively optimized adjustment parameter that modifies one or more of said differential equations.

176. The article of manufacture of claim 175, wherein said *in vitro* data is obtained from testing of a compound in one or more assays that generate data selected from the group consisting of cell, tissue, structure-activity relationship (SAR), and quantitative structure-activity relationship (QSAR) data.

177. The article of manufacture of claim 175, wherein said different compounds of a compound test set comprise compounds having diverse pharmacokinetic properties in said mammalian system of interest.

178. The article of manufacture of claim 174, wherein said input data comprises *in vivo* data from a first species of mammal and said mammalian system of interest comprises a second species of mammal, and wherein said selectively optimized adjustment parameter comprises a best fit value obtained by (i) assigning an initial value to a selected adjustment parameter of said simulation model, (ii) fitting a combination of *in vivo* data with said simulation model, said combination of *in vivo* data derived from testing of different compounds of a compound test set in said first species of mammal and said second species of mammal, (iii) selecting a best fit value for said selected adjustment parameter that, when assigned as an initial value to said selected adjustment parameter, permits said simulation model to predict said pharmacokinetic property of said compound when said input data comprises said *in vitro* data from said first species of mammal, and (iv) assigning said best fit value as a constant to said selected adjustment parameter so as to generate said selectively optimized adjustment parameter that modifies one or more of said differential equations.

179. The article of manufacture of claim 178, wherein said different compounds of a compound test set comprise compounds having diverse pharmacokinetic properties in said mammalian system of interest.

180. The article of manufacture of claim 160, wherein said selectively optimized adjustment parameter is for one or more of fluid absorption, flux, permeability, transport mechanism, transfer rate, and segment surface area.

181. The article of manufacture of claim 160, wherein said computer readable physiologic pharmacokinetic simulation model comprises at least two of said anatomical segments and a logic function module comprising a regional correlation

estimation function and a control statement for initiating said function, wherein said estimation function when initiated is capable of generating an estimated value for a selected pharmacokinetic property of said compound in a first anatomical segment when supplied with an input value corresponding to said selected pharmacokinetic property in a second anatomical segment and with a regional correlation coefficient for said selected pharmacokinetic property of said first and second anatomical segments.

182. A computer program product for simulating a pharmacokinetic property of a compound in a mammal of interest, and having computer readable program code input/output system, computer readable program code simulation engine, and computer readable program code physiologic pharmacokinetic simulation model of one or more anatomical segments of a selected mammalian system, said computer readable program code physiologic pharmacokinetic simulation model comprising differential equations for calculating as a function of time the change in (i) a physiological parameter of one or more of said segments and (ii) a pharmacokinetic property comprising an absorption parameter of a compound relative to a selected route of administration, barrier to absorption and sampling site of one or more of said segments, wherein one or more of said differential equations is modified by a selectively optimized adjustment parameter;

said computer readable program code input/output system, said computer readable program code simulation engine, and said computer readable program code physiologic pharmacokinetic simulation model being capable of working together to carry out the steps of:

(a) receiving through said computer readable program code input/output system input data comprising dose, permeability and solubility data for said compound for one or more segments of said mammalian system of interest; and

(b) applying said computer readable program code simulation engine and said computer readable program code physiologic pharmacokinetic simulation model to simulate a pharmacokinetic property comprising an absorption parameter of said compound relative to one or more segments of said mammalian system of interest.

183. The article of manufacture of claim 182, wherein said computer readable program code physiologic pharmacokinetic simulation model comprises at least two of said anatomical segments and a logic function model comprising a regional correlation estimation function and a control statement for initiating said function, wherein said estimation function when initiated is capable of generating an estimated value for a selected pharmacokinetic property of said compound in a first anatomical segment when supplied with an input value corresponding to said selected pharmacokinetic property in a second anatomical segment and with a regional correlation coefficient for said selected pharmacokinetic property of said first and second anatomical segments.

184. An article of manufacture comprising a computer readable medium having computer readable program code embodied therein for simulating a pharmacokinetic property of a compound in a mammal of interest, and having computer readable input/output system, computer readable simulation engine, and computer readable physiologic pharmacokinetic simulation model of at least two segments of a selected mammalian system of interest, said computer readable physiologic pharmacokinetic simulation model comprising as operably linked components:

(i) differential equations for calculating the change in one or more physiological parameters of first and second segments of said mammalian system of interest and the movement and disposition of said compound in said first and second segments as a function of time, and (ii) a logic function module having a regional correlation parameter estimation function and a control statement for initiating said function, wherein said estimation function when initiated is capable of generating an estimated value for a selected pharmacokinetic property comprising an absorption parameter of said compound in said first segment when supplied with an input value corresponding to said selected pharmacokinetic property of said compound in said second segment and with a regional correlation coefficient for said selected pharmacokinetic parameter of said first and second segments;

said computer readable input/output system, said computer readable simulation engine, and said computer readable physiologic pharmacokinetic simulation model being capable of working together to carry out the steps of:

(a) receiving through said computer readable input/output system input data comprising a pharmacokinetic property of said compound in said second segment of said mammalian system of interest; and

(b) applying said computer readable simulation engine and said computer readable physiologic pharmacokinetic simulation model to initiate said estimation function to estimate said pharmacokinetic property of said compound in said first segment of said mammalian system of interest.

185. The article of manufacture of claim 184, wherein said regional correlation estimation function comprises a function/transformation algorithm.

186. The article of manufacture of claim 185, wherein said function/transformation algorithm is selected from the group consisting of a polynomial, exponential, and logarithm.

187. The article of manufacture of claim 184, wherein said regional correlation coefficient comprises a best fit value that transforms said input data comprising said pharmacokinetic property of said compound in said second segment to an estimated pharmacokinetic property of said compound in said first segment.

188. The article of manufacture of claim 160 or 184, wherein said pharmacokinetic property is selected from the group consisting of absorption, distribution, metabolism, elimination and toxicity.

189. The article of manufacture of claim 184, wherein said pharmacokinetic parameter is selected from the group consisting of permeability, solubility, dissolution rate and transport mechanism.

190. The article of manufacture of claim 184, wherein said differential equations are selected from the group consisting of equations for fluid transit, fluid absorption, mass transit, mass dissolution, mass solubility, and mass absorption.

191. The article of manufacture of claim 184, wherein said mammalian system of interest is selected from the group consisting of gastrointestinal tract, liver, heart, kidney, eye, nose, lung, skin and brain.

192. The article of manufacture of claim 160 or 184, wherein said mammalian system of interest is human.

193. The article of manufacture of claim 184, wherein said input data comprises *in vitro* data.

194. The article of manufacture of claim 193, wherein said *in vitro* data is derived from testing of said compound in an assay that generates data selected from the group consisting of cell, tissue, physicochemical, structure-activity relationship (SAR) SAR, and quantitative structure-activity relationship (QSAR) QSAR data.

195. The article of manufacture of claim 184, wherein one or more of said differential equations is modified by a selectively optimized adjustment parameter.

196. A computer program product for simulating a pharmacokinetic property of a compound in a mammal of interest, and having computer readable program code input/output system, computer readable program code simulation engine, and computer readable program code physiologic pharmacokinetic simulation model of at least two segments of a selected mammalian system of interest, said computer readable program code physiologic pharmacokinetic simulation model comprising as operably linked components:

(i) differential equations for calculating the change in one or more physiological parameters of first and second segments of said mammalian system of interest and the movement and disposition of said compound in said first and second segments as a function of time, and (ii) a logic function module having a regional correlation parameter estimation function and a control statement for initiating said function, wherein said estimation function when initiated is capable of generating an estimated value for a selected pharmacokinetic property comprising an absorption parameter of said compound in said first segment when supplied with an input value corresponding to said selected pharmacokinetic property of said compound in said second segment and with a regional correlation coefficient for said selected pharmacokinetic parameter of said first and second segments;

said computer readable program code input/output system, said computer readable program code simulation engine, and said computer readable program code physiologic pharmacokinetic simulation model being capable of working together to carry out the steps of:

(a) receiving through said computer readable program code input/output system input data comprising a pharmacokinetic property of said compound in said second segment of said mammalian system of interest; and

(b) applying said computer readable program code simulation engine and said computer readable program code physiologic pharmacokinetic simulation model to initiate said estimation function to estimate said pharmacokinetic property of said compound in said first segment of said mammalian system of interest.

197. The computer program product of claim 196, wherein one or more of said differential equations is modified by a selectively optimized adjustment parameter.

198. An article of manufacture comprising a computer readable medium having embodied therein a computer readable physiologic pharmacokinetic simulation model of one or more anatomical segments of a selected mammalian system, said computer readable physiologic pharmacokinetic simulation model comprising differential equations for calculating as a function of time the change in (i) a physiological parameter of one or more of said segments and (ii) a pharmacokinetic property comprising an absorption parameter of a compound relative to a selected route of administration, barrier to absorption and sampling site of one or more of said segments, wherein one or more of said differential equations is modified by a selectively optimized adjustment parameter.

199. An article of manufacture comprising a computer readable medium having embodied therein a computer readable physiologic pharmacokinetic simulation model of at least two segments of a selected mammalian system of interest, said computer readable physiologic pharmacokinetic simulation model comprising as operably linked components:

(i) differential equations for calculating the change in one or more physiological parameters of first and second segments of said mammalian system of interest and the movement and disposition of said compound in said first and second segments as a function of time, and (ii) a logic function module having a regional correlation parameter estimation function and a control statement for initiating said function, wherein said estimation function when initiated is capable of generating an estimated value for a selected pharmacokinetic property comprising an absorption parameter of said compound in said first segment when supplied with an input value corresponding to said selected pharmacokinetic property of said compound in said second segment and with a regional correlation coefficient for said selected pharmacokinetic parameter of said first and second segments.

FIG. 1

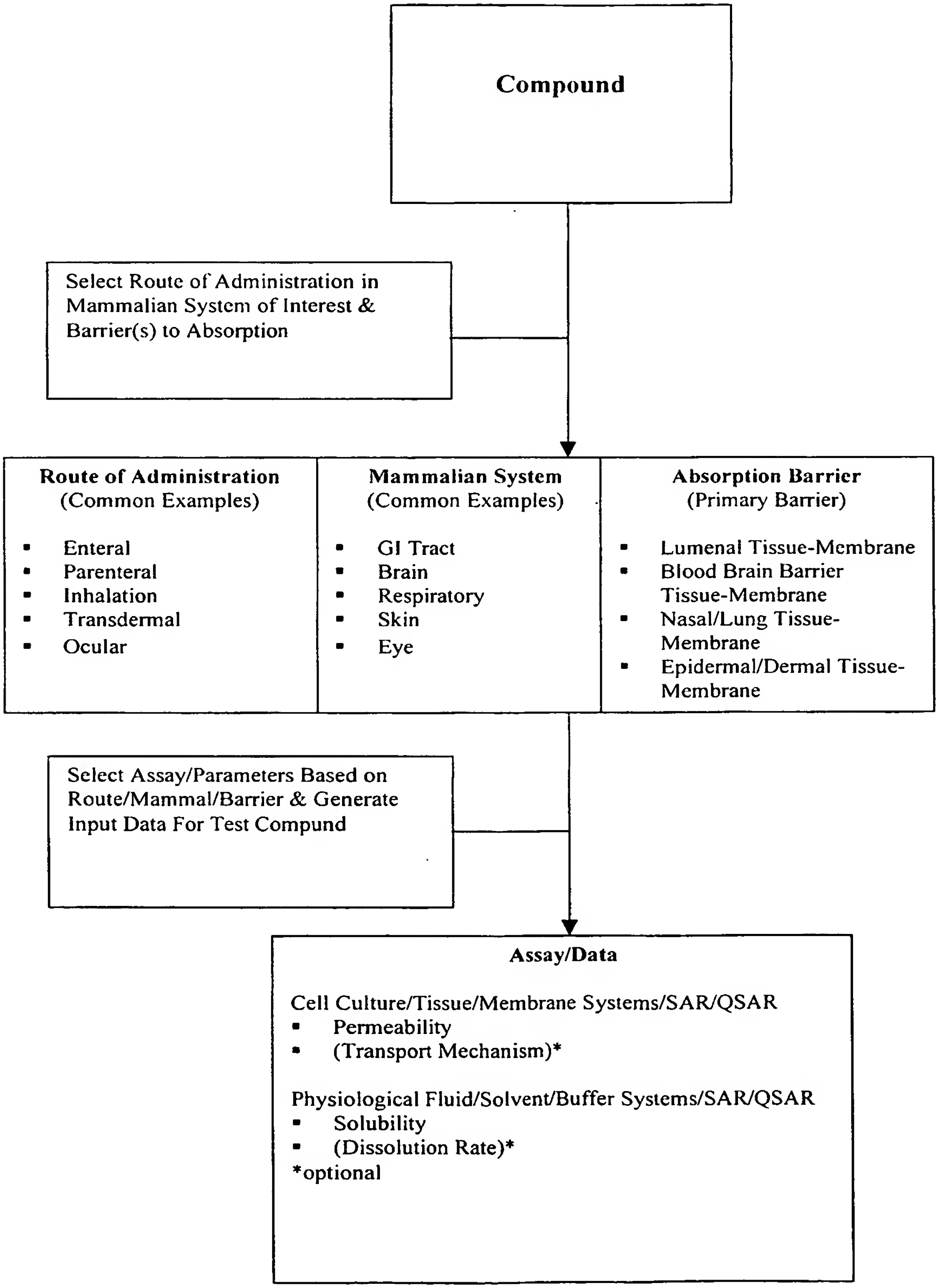

Compound
Select Route of Administration in Mammalian System of Interest & Barrier(s) to Absorption
Route of Administration (Common Examples)
- Enteral
- Parenteral
- Inhalation
- Transdermal
- Ocular
Mammalian System (Common Examples)
- GI Tract
- Brain
- Respiratory
- Skin
- Eye
Absorption Barrier (Primary Barrier)
- Lumenal Tissue-Membrane
- Blood Brain Barrier Tissue-Membrane
- Nasal/Lung Tissue-Membrane
- Epidermal/Dermal Tissue-Membrane
Select Assay/Parameters Based on Route/Mammal/Barrier & Generate Input Data For Test Compund
Assay/Data
Cell Culture/Tissue/Membrane Systems/SAR/QSAR
- Permeability
- (Transport Mechanism)*
Physiological Fluid/Solvent/Buffer Systems/SAR/QSAR
- Solubility
- (Dissolution Rate)*
*optional

FIG. 2

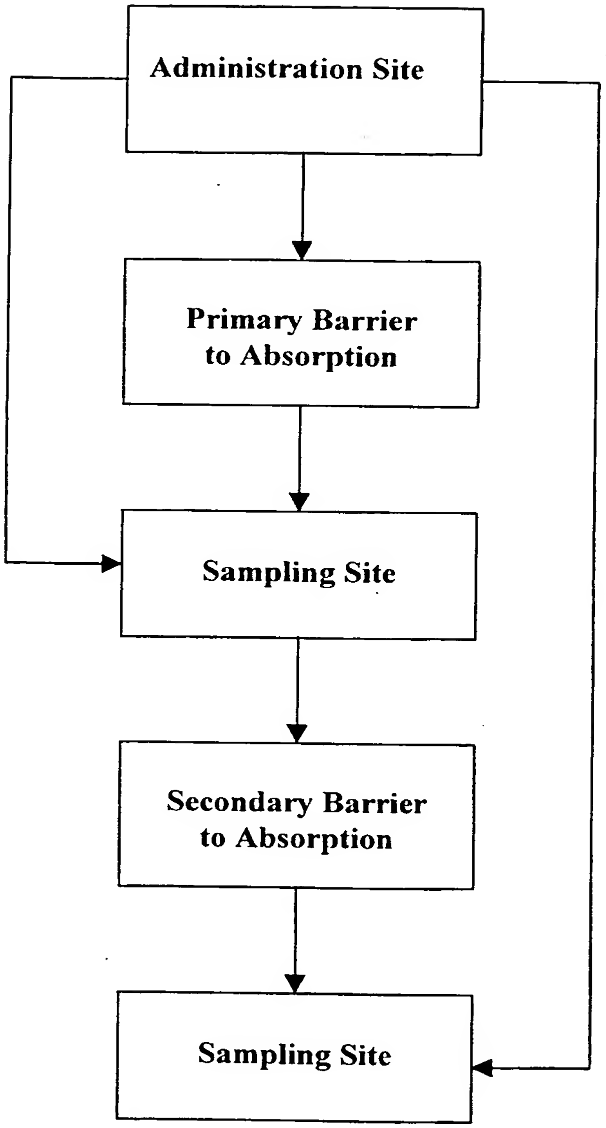

Administration Site
Primary Barrier to Absorption
Sampling Site
Secondary Barrier to Absorption
Sampling Site

FIG. 3

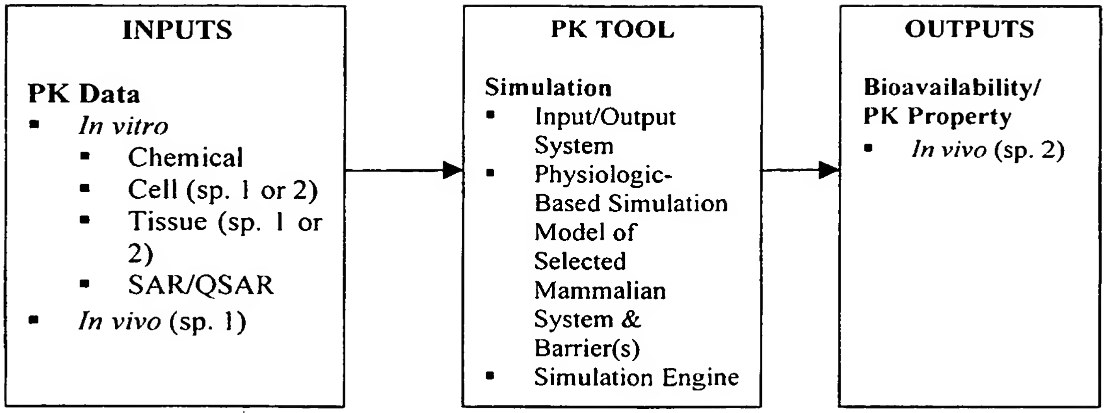

INPUTS
PK Data
In vitro
Chemical
Cell (sp. 1 or 2)
Tissue (sp. 1 or 2)
SAR/QSAR
In vivo (sp. 1)
PK TOOL
Simulation
Input/Output System
Physiologic-Based Simulation Model of Selected Mammalian System & Barrier(s)
Simulation Engine
OUTPUTS
Bioavailability/ PK Property
In vivo (sp. 2)

FIG. 4

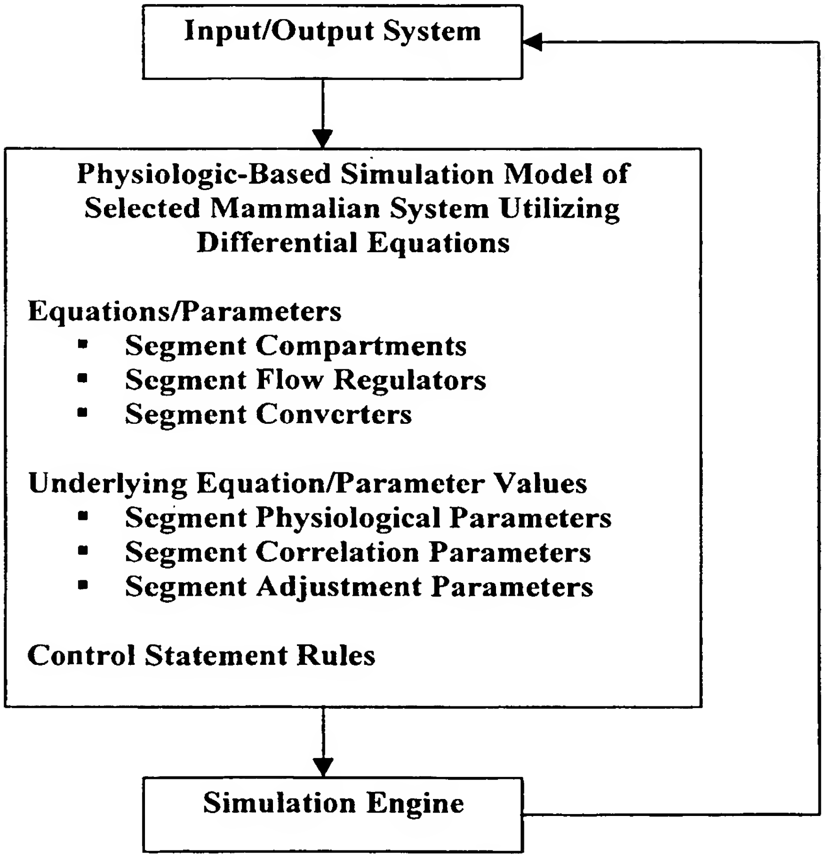

Input/Output System
Physiologic-Based Simulation Model of Selected Mammalian System Utilizing Differential Equations
Equations/Parameters
Segment Compartments
Segment Flow Regulators
Segment Converters
Underlying Equation/Parameter Values
Segment Physiological Parameters
Segment Correlation Parameters
Segment Adjustment Parameters
Control Statement Rules
Simulation Engine

FIG. 5

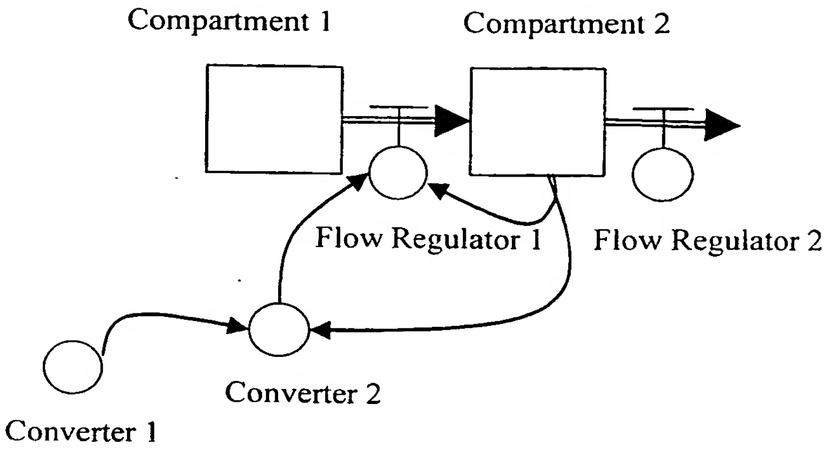

Compartment 1
Compartment 2
Flow Regulator 1
Flow Regulator 2
Converter 2
Converter 1

FIG. 6

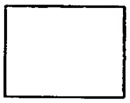
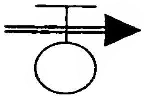
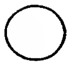
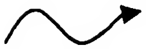
Symbol	**Name**	**Time-Dependent Function**
	Compartment	Equation or value for amount of substance stored.
	Flow Regulator	Rate equation for amount of substance transferred.
	Converter	Equation or pre-defined value for (i) input into flow regulator; (ii) input into another converter; and/or (iii) storing value.
	Input Link	Directs input values.

FIG. 7

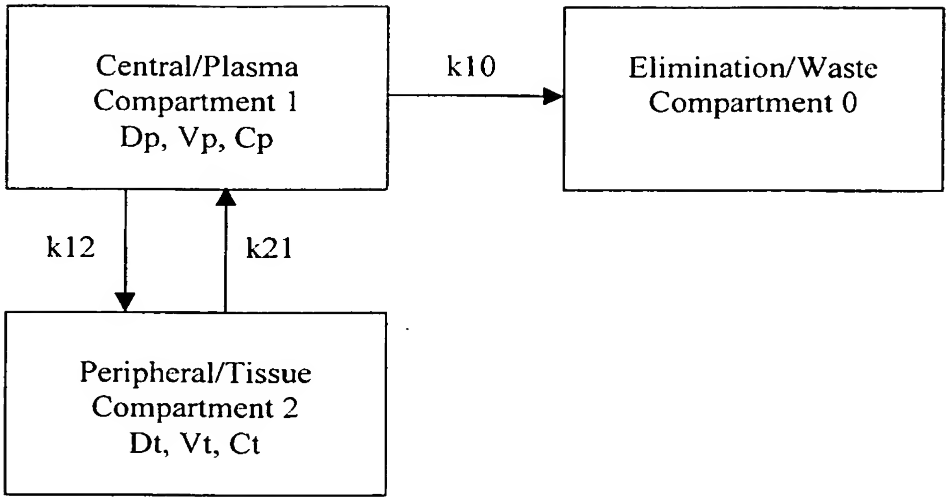

Central/Plasma
Compartment 1
Dp, Vp, Cp
k10
Elimination/Waste
Compartment 0
k12
k21
Peripheral/Tissue
Compartment 2
Dt, Vt, Ct

FIG. 8

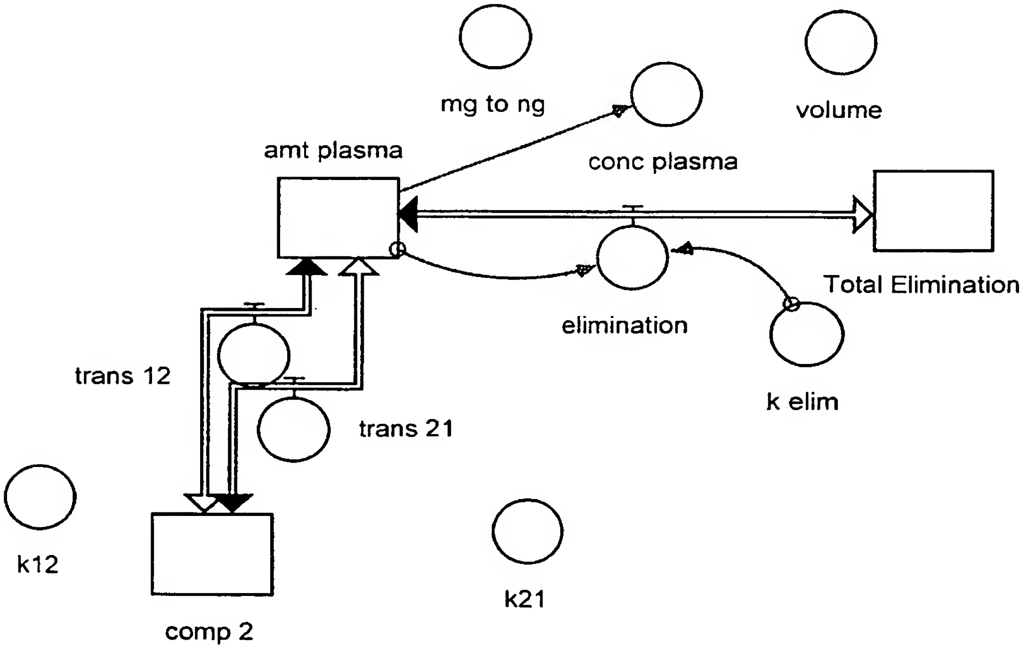

mg to ng
volume
conc plasma
amt plasma
Total Elimination
elimination
trans 12
trans 21
k elim
k12
k21
comp 2

FIG. 9

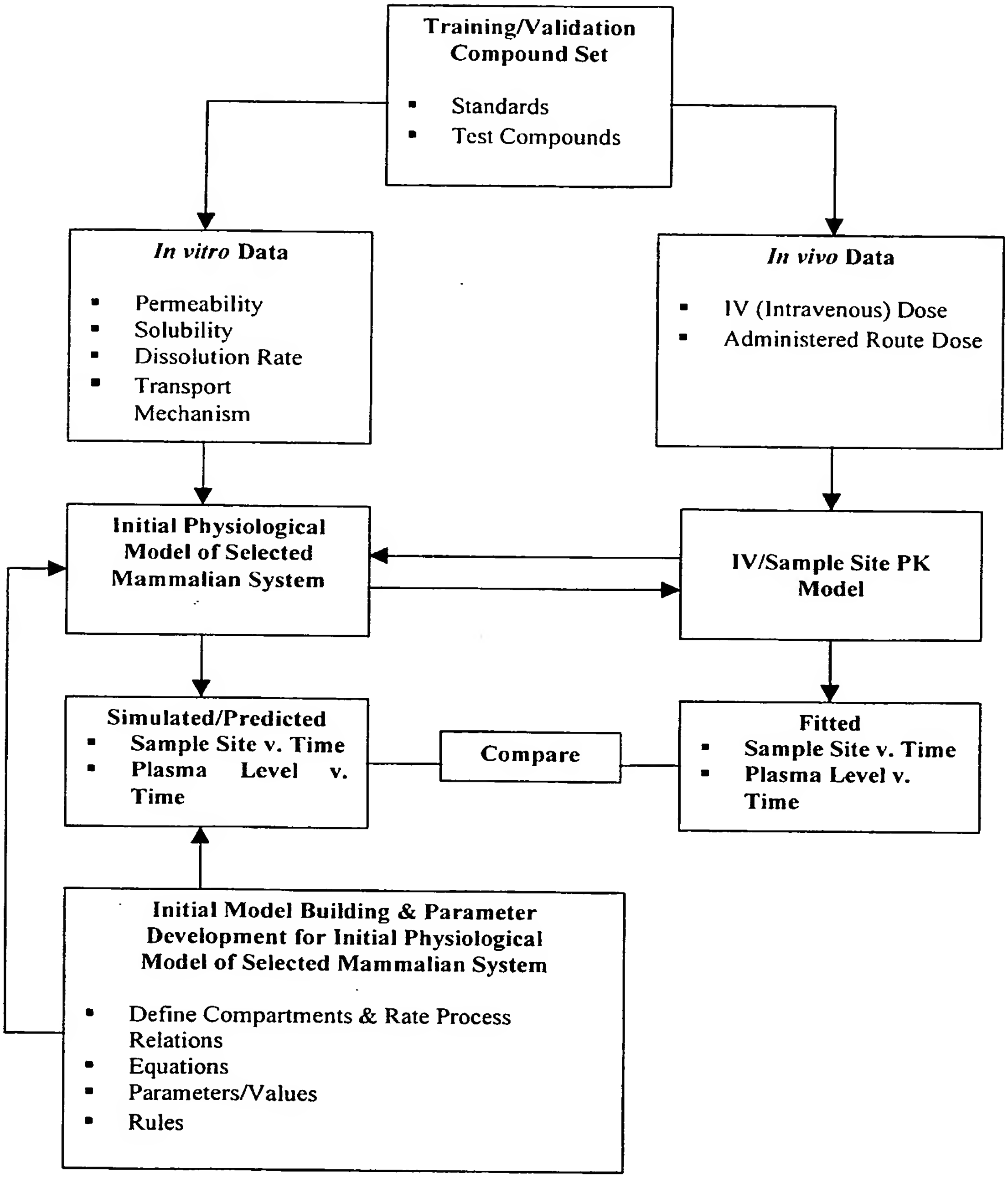

Training/Validation Compound Set
Standards
Test Compounds
In vitro Data
Permeability
Solubility
Dissolution Rate
Transport Mechanism
In vivo Data
IV (Intravenous) Dose
Administered Route Dose
Initial Physiological Model of Selected Mammalian System
IV/Sample Site PK Model
Simulated/Predicted
Sample Site v. Time
Plasma Level v. Time
Compare
Fitted
Sample Site v. Time
Plasma Level v. Time
Initial Model Building & Parameter Development for Initial Physiological Model of Selected Mammalian System
Define Compartments & Rate Process Relations
Equations
Parameters/Values
Rules

FIG. 10

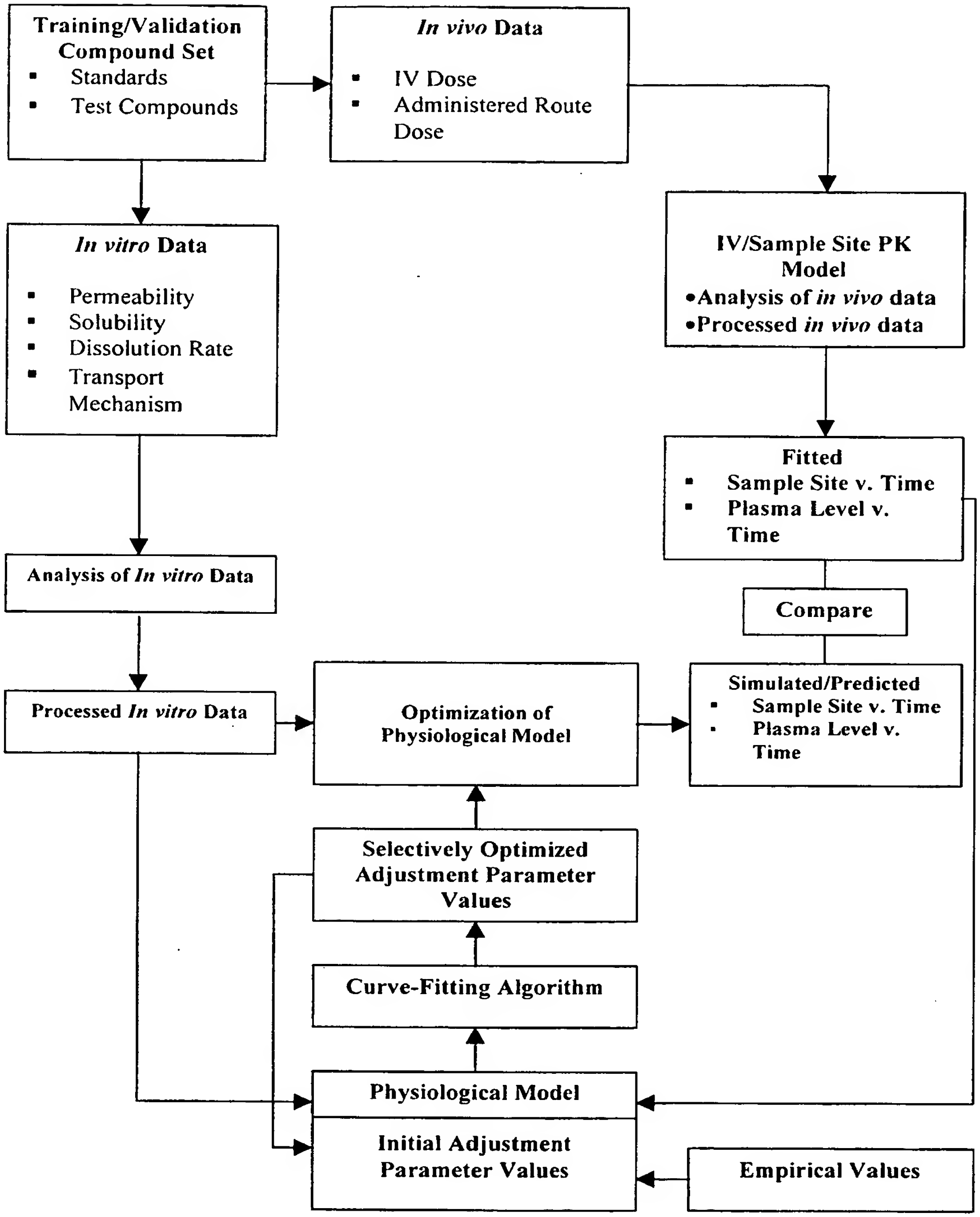
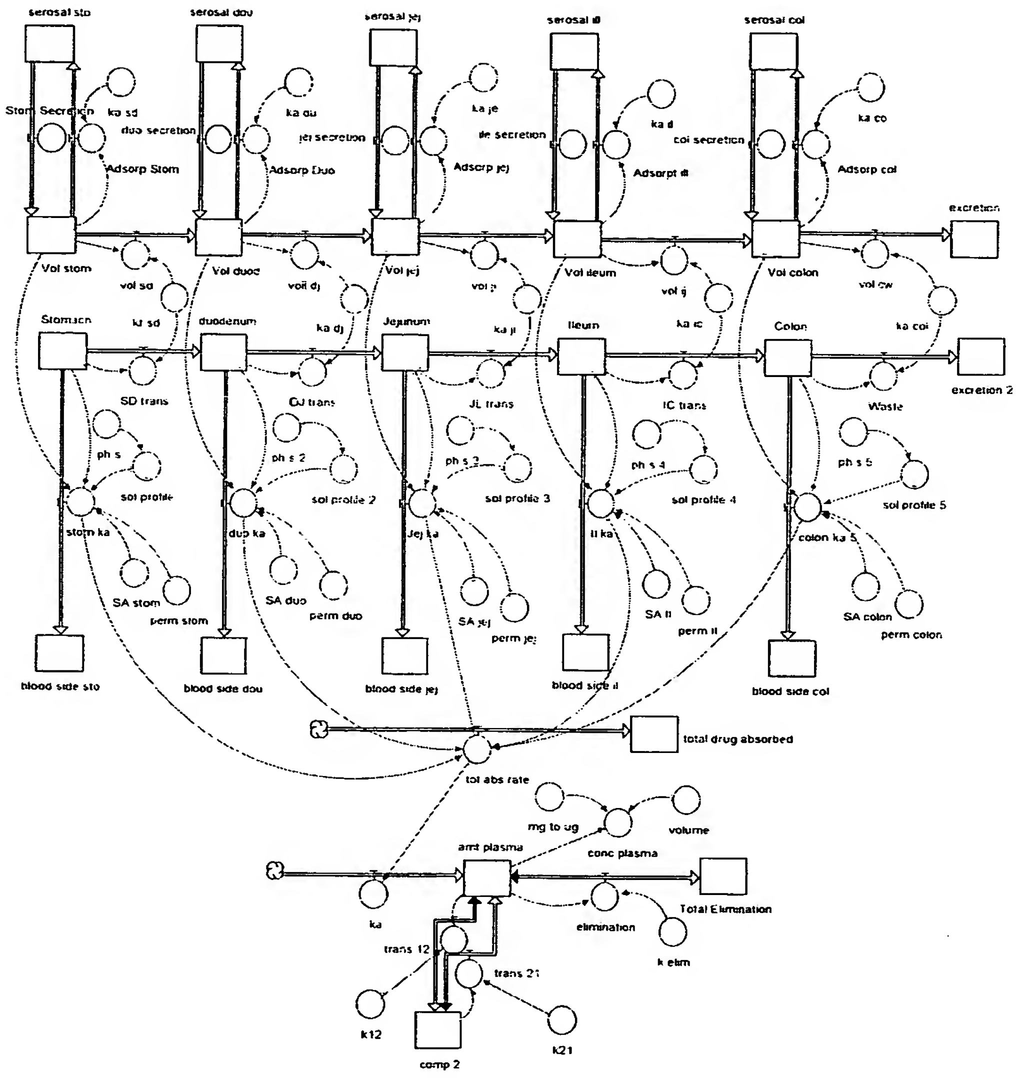

Training/Validation Compound Set
Standards
Test Compounds
In vivo Data
IV Dose
Administered Route Dose
In vitro Data
Permeability
Solubility
Dissolution Rate
Transport Mechanism
IV/Sample Site PK Model
•Analysis of *in vivo* data
•Processed *in vivo* data
Fitted
Sample Site v. Time
Plasma Level v. Time
Analysis of *In vitro* Data
Compare
Processed *In vitro* Data
Optimization of Physiological Model
Simulated/Predicted
Sample Site v. Time
Plasma Level v. Time
Selectively Optimized Adjustment Parameter Values
Curve-Fitting Algorithm
Physiological Model
Initial Adjustment Parameter Values
Empirical Values

FIG. 11



serosal sto
serosal dou
serosal jej
serosal il
serosal col
Stom Secretion
ka sd
duo secretion
ka du
jej secretion
ka je
ile secretion
ka il
col secretion
ka co
Adsorp Stom
Adsorp Duo
Adsorp jej
Adsorpt il
Adsorp col
excretion
Vol stom
Vol duod
Vol jej
Vol ileum
Vol colon
vol sd
voll dj
vol ji
vol ij
vol cw
Stomach
kl sd
duodenum
ka dj
Jejunum
ka ji
Ileum
ka ic
Colon
ka col
excretion 2
SD trans
DJ trans
JL trans
IC trans
Waste
ph s
ph s 2
ph s 3
ph s 4
ph s 5
sol profile
sol profile 2
sol profile 3
sol profile 4
sol profile 5
stom ka
duo ka
Jej ka
il ka
colon ka 5
SA stom
perm stom
SA duo
perm duo
SA jej
perm jej
SA il
perm il
SA colon
perm colon
blood side sto
blood side dou
blood side jej
blood side il
blood side col
total drug absorbed
tot abs rate
mg to ug
volume
conc plasma
amt plasma
ka
Total Elimination
elimination
k elim
trans 12
trans 21
k12
k21
comp 2

FIG. 12

Mass-Volume GI Tract Model

- **GI Segment Compartments**
  - Fluid Volume
  - Fluid Absorption
  - Insoluble Mass
  - Soluble Mass Absorption
- **GI Segment Flow Regulators**
  - Fluid Volume Absorption Rate
  - Fluid Volume Secretion Rate
  - Fluid Volume GI Transit Rate
  - Insoluble Mass GI Transit Rate
  - Soluble Mass Absorption Rate
- **GI Segment Converters**
  - Rate Constant
  - pH
  - Solubility
  - Surface Area
  - Permeability

FIG. 13

Mass-Volume GI Tract Model

- **GI Segment Compartments & *Flow Regulators***
  - **Fluid Volume**
    - *Fluid Volume Absorption Rate*
    - *Fluid Volume Secretion Rate*
    - *Fluid Volume GI Transit Rate*
  - **Fluid Volume Absorption**
    - *Fluid Volume Absorption Rate*
    - *Fluid Volume Secretion Rate*
  - **Insoluble Mass**
    - *Insoluble Mass GI Transit Rate*
    - *Soluble Mass Absorption Rate*
  - **Soluble Mass Absorption**
    - *Soluble Mass Absorption Rate*

FIG. 14

Mass-Volume GI Tract Model

- **GI Segment Flow Regulators &** ***Converters***
  - **Fluid Volume Absorption Rate**
    - *Fluid Volume Absorption Rate Constant*
  - **Fluid Volume Secretion Rate**
    - *Fluid Volume Secretion Rate Constant*
  - **Fluid Volume GI Transit Rate**
    - *Fluid Volume GI Transit Rate Constant*
  - **Insoluble Mass GI Transit Rate**
    - *Insoluble Mass GI Transit Rate Constant*
  - **Soluble Mass Absorption Rate**
    - *Fluid Volume*
    - *Insoluble Mass*
    - *Mass Solubility Profile*
      - *pH*
    - *Permeability*
    - *Surface Area*

FIG. 15

Mass-Volume GI Tract Model

- **GI Segment Converters**
  - Rate Constant
  - pH
  - Solubility
  - Surface Area
  - Permeability

FIG. 16

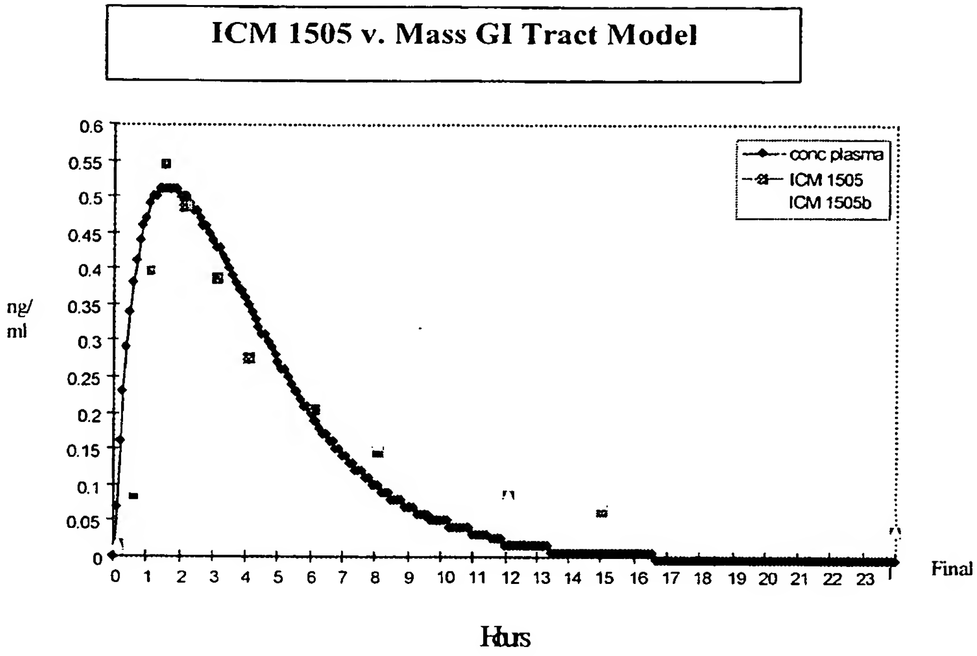

ICM 1505 v. Mass GI Tract Model
ng/ ml
0.6
0.55
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
conc plasma
ICM 1505
ICM 1505b
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
Final
Hours

FIG. 17

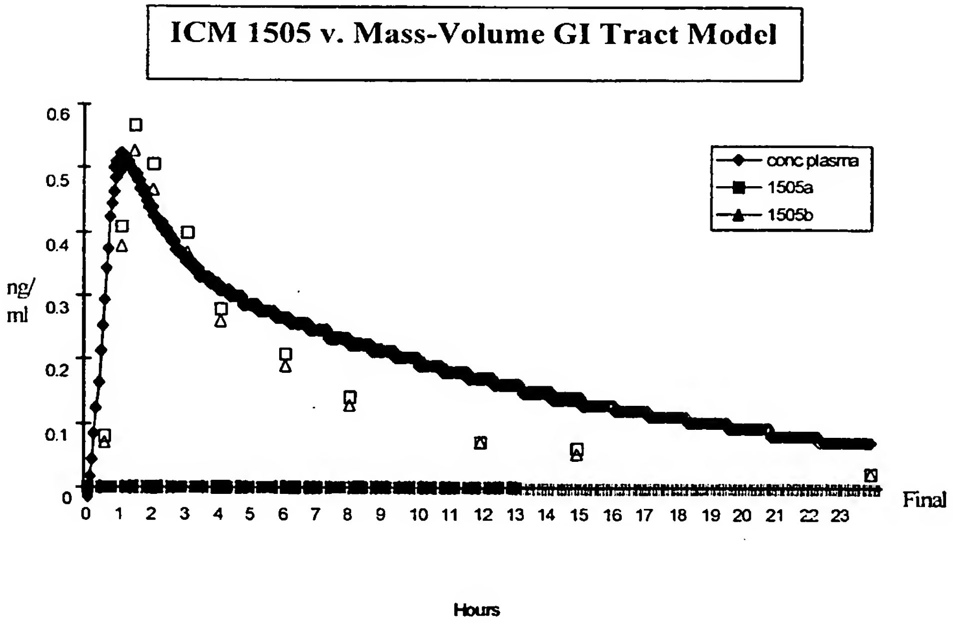

ICM 1505 v. Mass-Volume GI Tract Model
ng/ ml
0.6
0.5
0.4
0.3
0.2
0.1
0
conc plasma
1505a
1505b
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
Final
Hours

FIG. 18

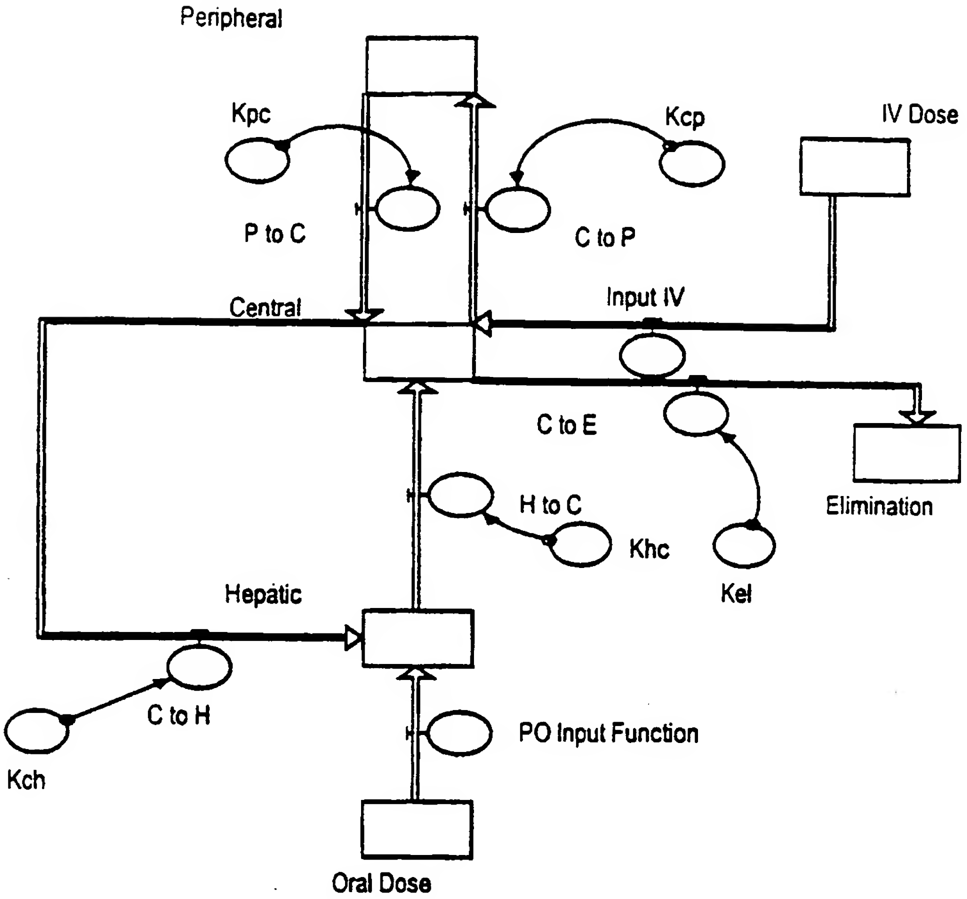

Peripheral
Kpc
Kcp
IV Dose
P to C
C to P
Input IV
Central
C to E
Elimination
H to C
Khc
Kel
Hepatic
C to H
PO Input Function
Kch
Oral Dose

FIG. 19

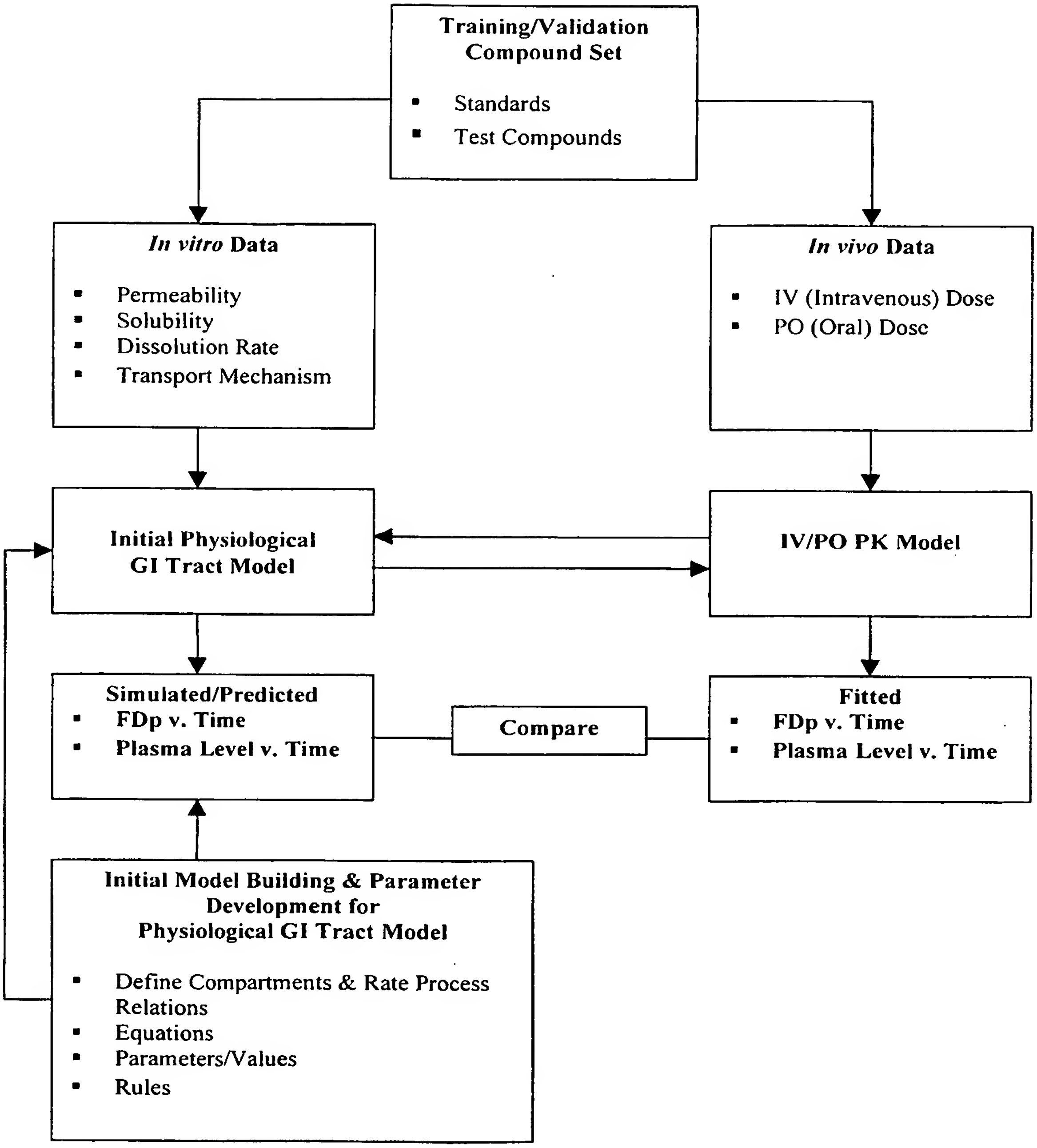

Training/Validation Compound Set
Standards
Test Compounds
In vitro Data
Permeability
Solubility
Dissolution Rate
Transport Mechanism
In vivo Data
IV (Intravenous) Dose
PO (Oral) Dose
Initial Physiological GI Tract Model
IV/PO PK Model
Simulated/Predicted
FDp v. Time
Plasma Level v. Time
Compare
Fitted
FDp v. Time
Plasma Level v. Time
Initial Model Building & Parameter Development for Physiological GI Tract Model
Define Compartments & Rate Process Relations
Equations
Parameters/Values
Rules

FIG. 20

Gastrointestinal Transit

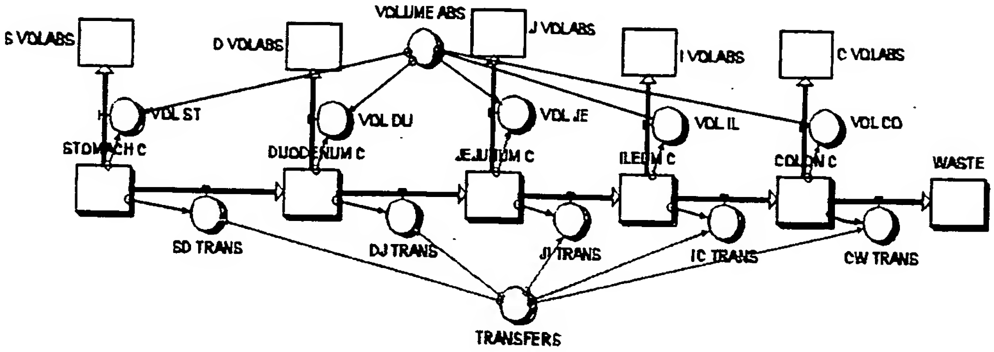

S VOLABS
D VOLABS
VOLUME ABS
J VOLABS
I VOLABS
C VOLABS
VOL ST
VOL DU
VOL JE
VOL IL
VOL CO
STOMACH C
DUODENUM C
JEJUNUM C
ILEUM C
COLON C
WASTE
SD TRANS
DJ TRANS
JI TRANS
IC TRANS
CW TRANS
TRANSFERS

FIG. 21
pH Dependent Solubility and Dissolution

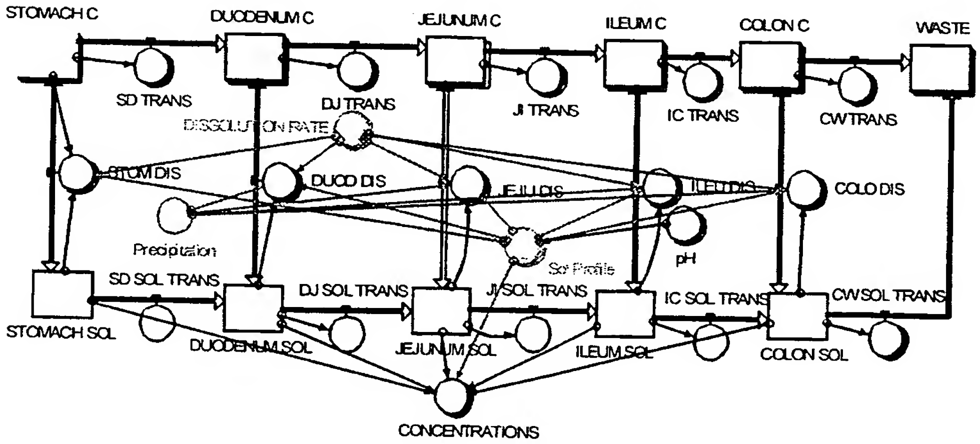

STOMACH C
DUODENUM C
JEJUNUM C
ILEUM C
COLON C
WASTE
SD TRANS
DJ TRANS
JI TRANS
IC TRANS
CW TRANS
DISSOLUTION RATE
STOM DIS
DUOD DIS
JEJU DIS
ILEU DIS
COLO DIS
Precipitation
pH
Sol Profile
SD SOL TRANS
DJ SOL TRANS
JI SOL TRANS
IC SOL TRANS
CW SOL TRANS
STOMACH SOL
DUODENUM SOL
JEJUNUM SOL
ILEUM SOL
COLON SOL
CONCENTRATIONS

FIG. 22

Absorption

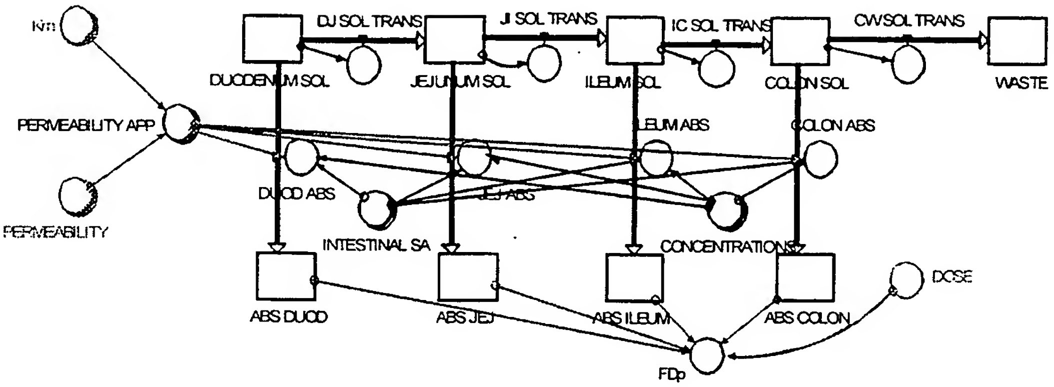

Km
DJ SOL TRANS
JI SOL TRANS
IC SOL TRANS
CW SOL TRANS
DUODENUM SOL
JEJUNUM SOL
ILEUM SOL
COLON SOL
WASTE
PERMEABILITY APP
ILEUM ABS
COLON ABS
DUOD ABS
JEJ ABS
PERMEABILITY
INTESTINAL SA
CONCENTRATIONS
DOSE
ABS DUOD
ABS JEJ
ABS ILEUM
ABS COLON
FDp

FIG. 23

GI Tract –Intestinal Model

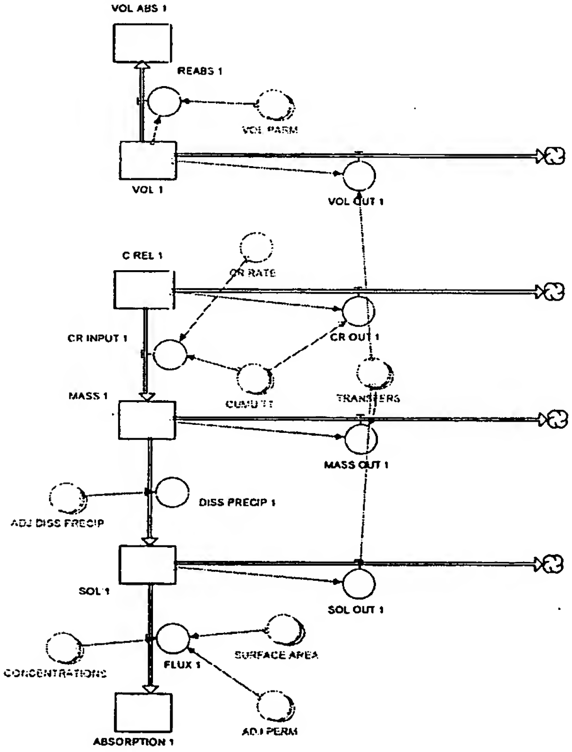

VOL ABS 1
REABS 1
VOL PARM
VOL 1
VOL OUT 1
C REL 1
CR RATE
CR INPUT 1
CR OUT 1
CUMUTT
TRANSFERS
MASS 1
MASS OUT 1
DISS PRECIP 1
ADJ DISS PRECIP
SOL 1
SOL OUT 1
SURFACE AREA
FLUX 1
CONCENTRATIONS
ABSORPTION 1
ADJ PERM

FIG. 24

GI Tract-Intestinal Model (without converters, ghosts or connectors)

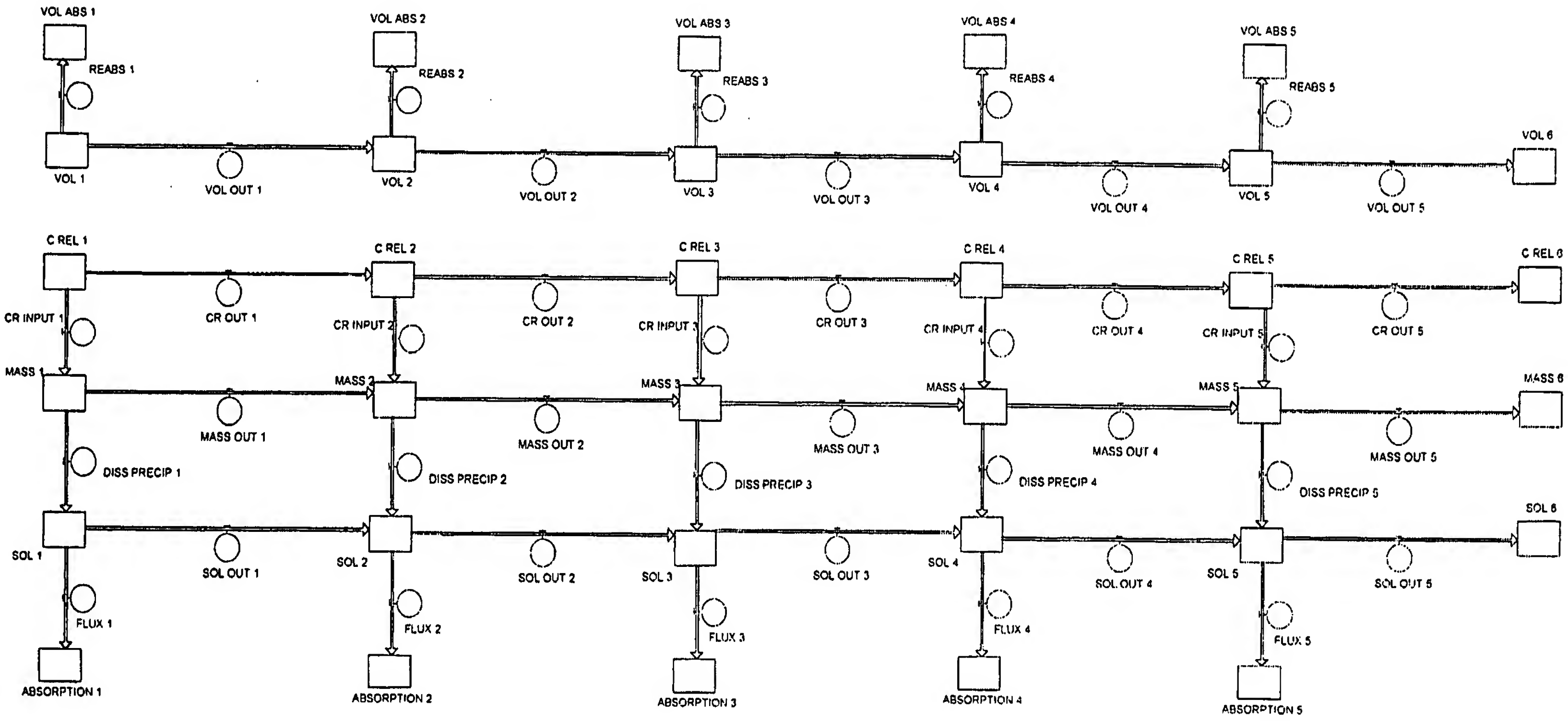

VOL ABS 1
REABS 1
VOL 1
VOL OUT 1
VOL ABS 2
REABS 2
VOL 2
VOL OUT 2
VOL ABS 3
REABS 3
VOL 3
VOL OUT 3
VOL ABS 4
REABS 4
VOL 4
VOL OUT 4
VOL ABS 5
REABS 5
VOL 5
VOL OUT 5
VOL 6
C REL 1
CR INPUT 1
CR OUT 1
C REL 2
CR INPUT 2
CR OUT 2
C REL 3
CR INPUT 3
CR OUT 3
C REL 4
CR INPUT 4
CR OUT 4
C REL 5
CR INPUT 5
CR OUT 5
C REL 6
MASS 1
MASS OUT 1
DISS PRECIP 1
MASS 2
MASS OUT 2
DISS PRECIP 2
MASS 3
MASS OUT 3
DISS PRECIP 3
MASS 4
MASS OUT 4
DISS PRECIP 4
MASS 5
MASS OUT 5
DISS PRECIP 5
MASS 6
SOL 1
SOL OUT 1
FLUX 1
ABSORPTION 1
SOL 2
SOL OUT 2
FLUX 2
ABSORPTION 2
SOL 3
SOL OUT 3
FLUX 3
ABSORPTION 3
SOL 4
SOL OUT 4
FLUX 4
ABSORPTION 4
SOL 5
SOL OUT 5
FLUX 5
ABSORPTION 5
SOL 6

FIG. 25

GI Tract-Intestinal Model

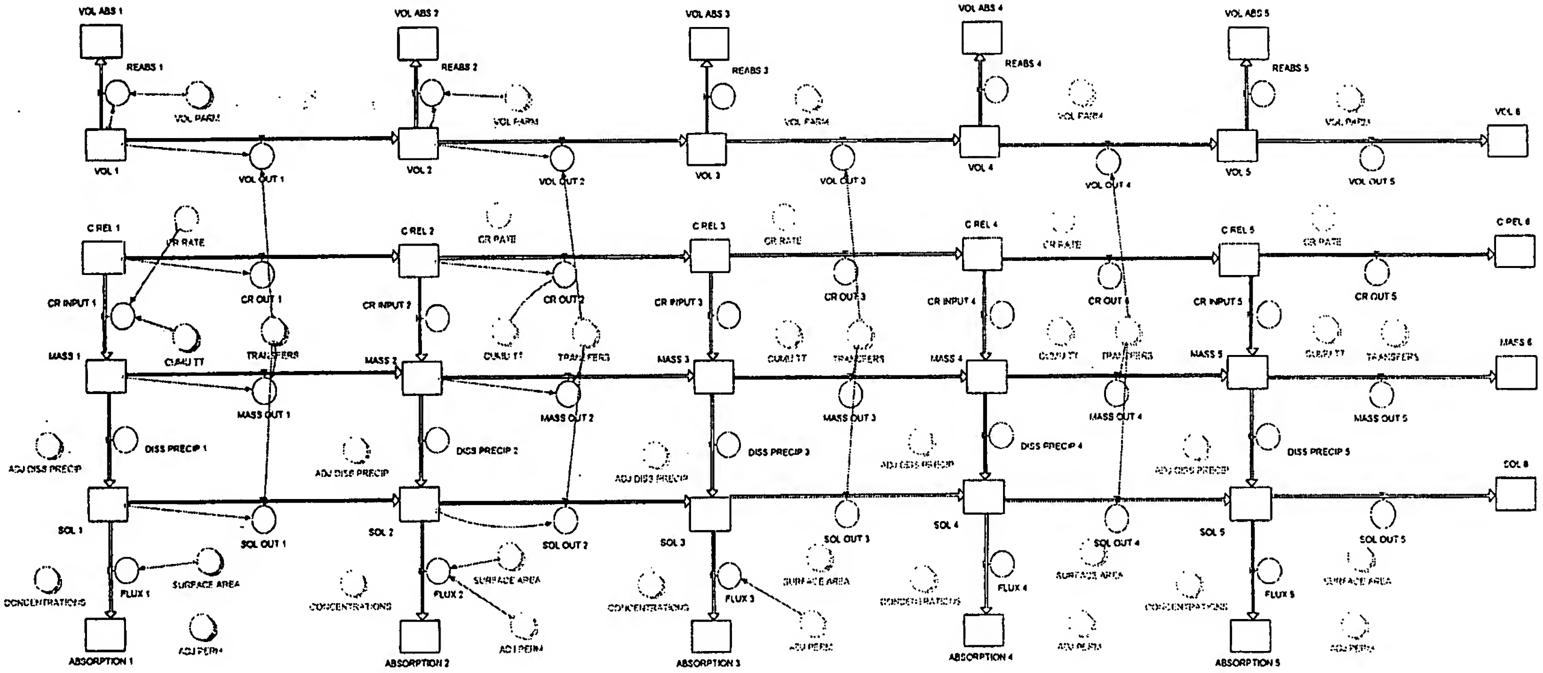

VOL ABS 1
REABS 1
VOL PARM
VOL 1
VOL OUT 1
VOL ABS 2
REABS 2
VOL PARM
VOL 2
VOL OUT 2
VOL ABS 3
REABS 3
VOL PARM
VOL 3
VOL OUT 3
VOL ABS 4
REABS 4
VOL PARM
VOL 4
VOL OUT 4
VOL ABS 5
REABS 5
VOL PARM
VOL 5
VOL OUT 5
VOL 6
C REL 1
CR RATE
CR INPUT 1
CR OUT 1
C REL 2
CR RATE
CR INPUT 2
CR OUT 2
C REL 3
CR RATE
CR INPUT 3
CR OUT 3
C REL 4
CR RATE
CR INPUT 4
CR OUT 4
C REL 5
CR RATE
CR INPUT 5
CR OUT 5
C REL 6
CUMU TT
TRANSFERS
CUMU TT
TRANSFERS
CUMU TT
TRANSFERS
CUMU TT
TRANSFERS
CUMU TT
TRANSFERS
MASS 1
MASS OUT 1
MASS 2
MASS OUT 2
MASS 3
MASS OUT 3
MASS 4
MASS OUT 4
MASS 5
MASS OUT 5
MASS 6
DISS PRECIP 1
ADJ DISS PRECIP
DISS PRECIP 2
ADJ DISS PRECIP
DISS PRECIP 3
ADJ DISS PRECIP
DISS PRECIP 4
ADJ DISS PRECIP
DISS PRECIP 5
ADJ DISS PRECIP
SOL 1
SOL OUT 1
SOL 2
SOL OUT 2
SOL 3
SOL OUT 3
SOL 4
SOL OUT 4
SOL 5
SOL OUT 5
SOL 6
FLUX 1
SURFACE AREA
CONCENTRATIONS
ADJ PERM
ABSORPTION 1
FLUX 2
SURFACE AREA
CONCENTRATIONS
ADJ PERM
ABSORPTION 2
FLUX 3
SURFACE AREA
CONCENTRATIONS
ADJ PERM
ABSORPTION 3
FLUX 4
SURFACE AREA
CONCENTRATIONS
ADJ PERM
ABSORPTION 4
FLUX 5
SURFACE AREA
CONCENTRATIONS
ADJ PERM
ABSORPTION 5

FIG. 26

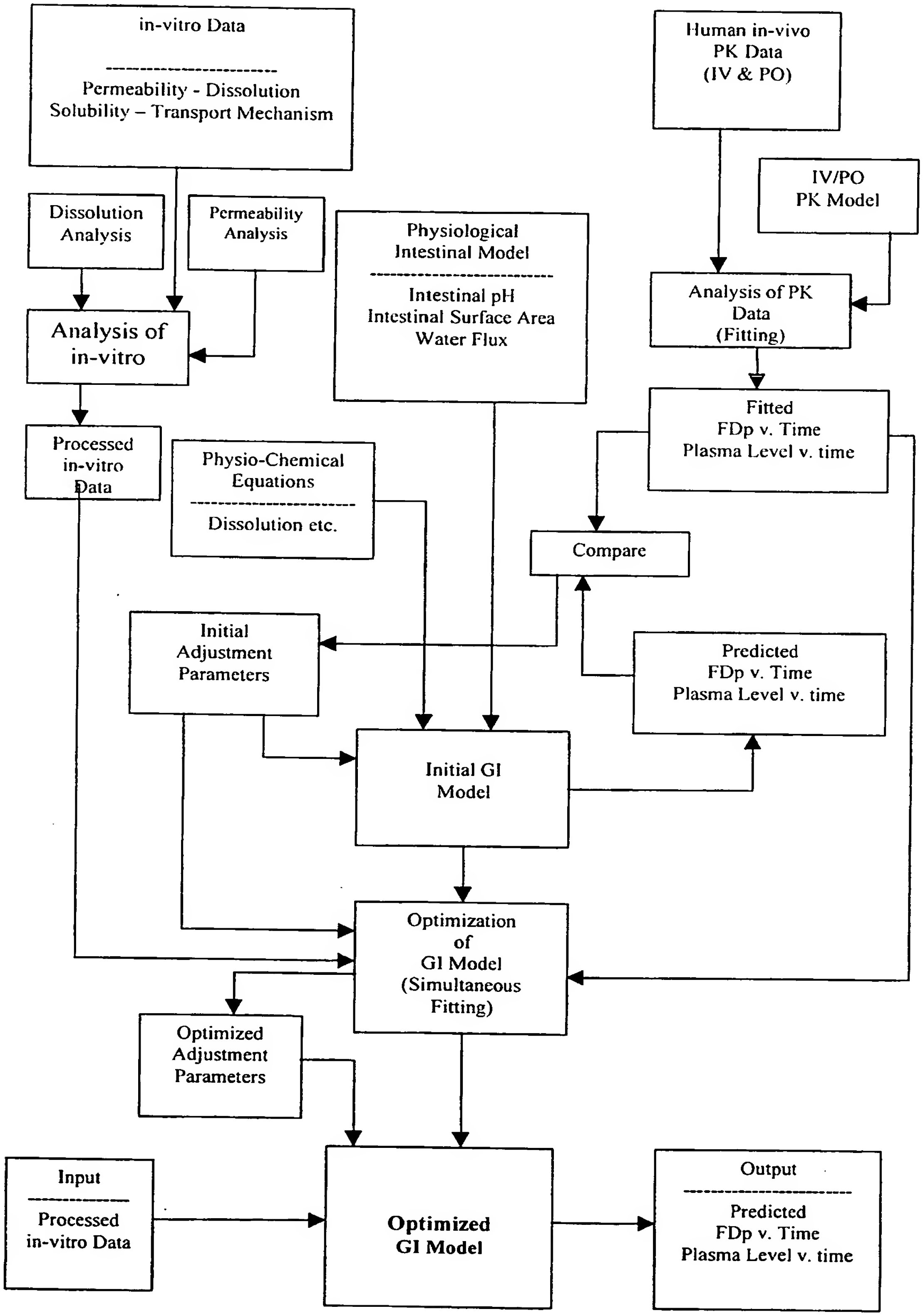

in-vitro Data
Permeability - Dissolution
Solubility – Transport Mechanism
Human in-vivo
PK Data
(IV & PO)
IV/PO
PK Model
Dissolution
Analysis
Permeability
Analysis
Physiological
Intestinal Model
Intestinal pH
Intestinal Surface Area
Water Flux
Analysis of PK
Data
(Fitting)
Analysis of
in-vitro
Fitted
FDp v. Time
Plasma Level v. time
Processed
in-vitro
Data
Physio-Chemical
Equations
Dissolution etc.
Compare
Initial
Adjustment
Parameters
Predicted
FDp v. Time
Plasma Level v. time
Initial GI
Model
Optimization
of
GI Model
(Simultaneous
Fitting)
Optimized
Adjustment
Parameters
Input
Processed
in-vitro Data
Optimized
GI Model
Output
Predicted
FDp v. Time
Plasma Level v. time

FIG. 27

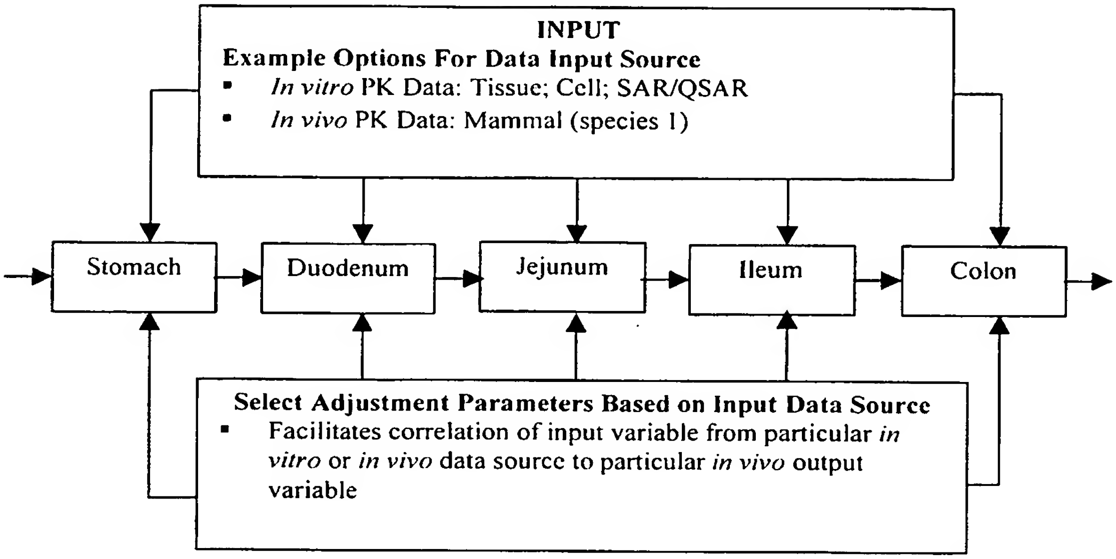

INPUT
Example Options For Data Input Source
In vitro PK Data: Tissue; Cell; SAR/QSAR
In vivo PK Data: Mammal (species 1)
Stomach
Duodenum
Jejunum
Ileum
Colon
Select Adjustment Parameters Based on Input Data Source
Facilitates correlation of input variable from particular in vitro or in vivo data source to particular in vivo output variable

FIG. 28

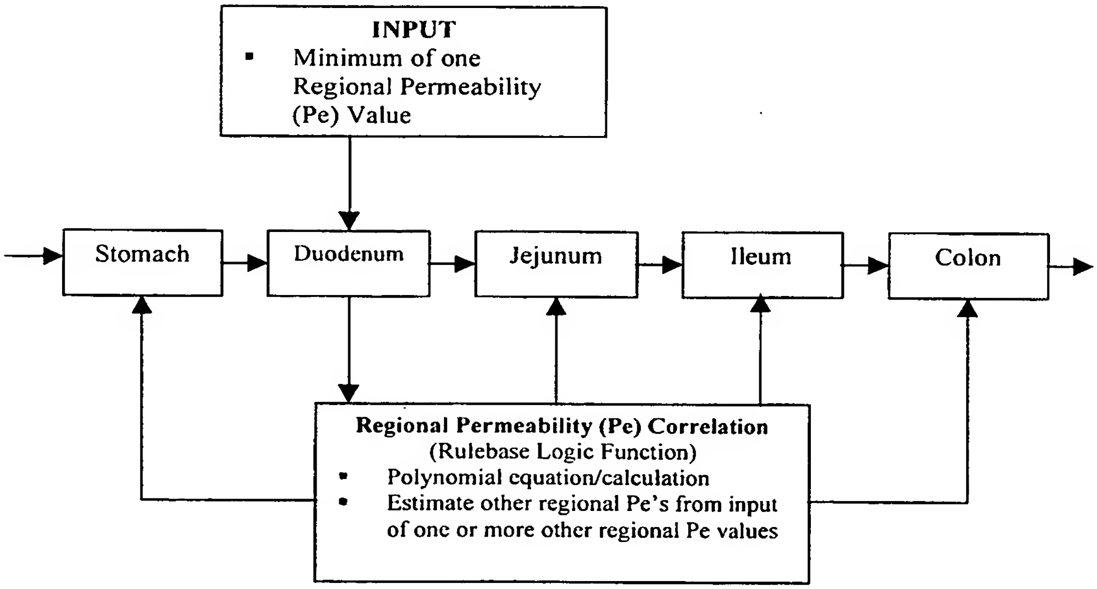

INPUT
Minimum of one Regional Permeability (Pe) Value
Stomach
Duodenum
Jejunum
Ileum
Colon
Regional Permeability (Pe) Correlation
(Rulebase Logic Function)
Polynomial equation/calculation
Estimate other regional Pe's from input of one or more other regional Pe values

FIG. 29

Parameters

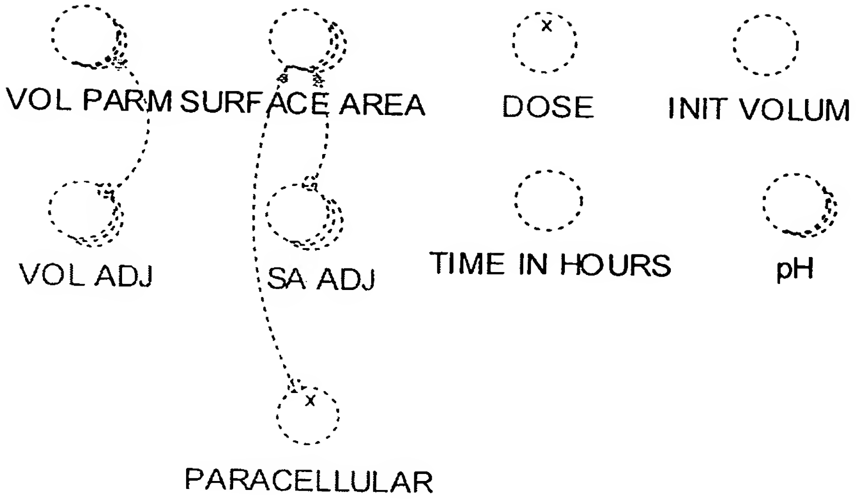

VOL PARM
SURFACE AREA
DOSE
INIT VOLUM
VOL ADJ
SA ADJ
TIME IN HOURS
pH
PARACELLULAR

FIG. 30

Transit Time

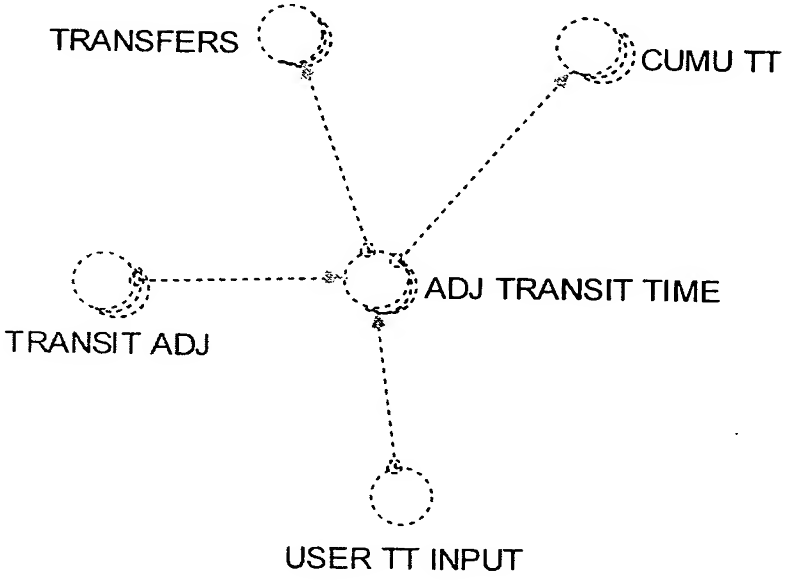

TRANSFERS
CUMU TT
ADJ TRANSIT TIME
TRANSIT ADJ
USER TT INPUT

FIG. 31

Permeability Calculation

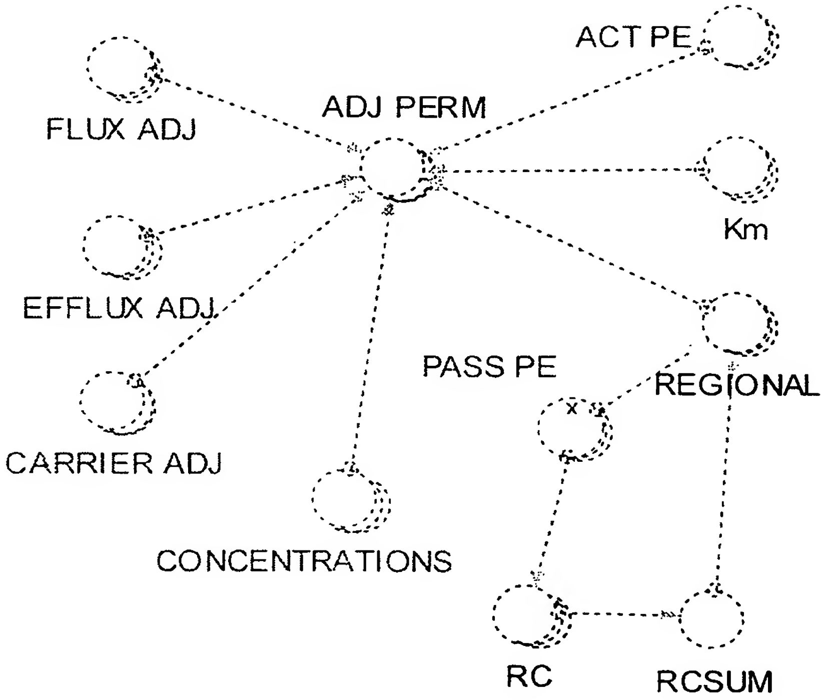

ACT PE
FLUX ADJ
ADJ PERM
Km
EFFLUX ADJ
PASS PE
REGIONAL
CARRIER ADJ
CONCENTRATIONS
RC
RCSUM

FIG. 32

Solubility Calculation

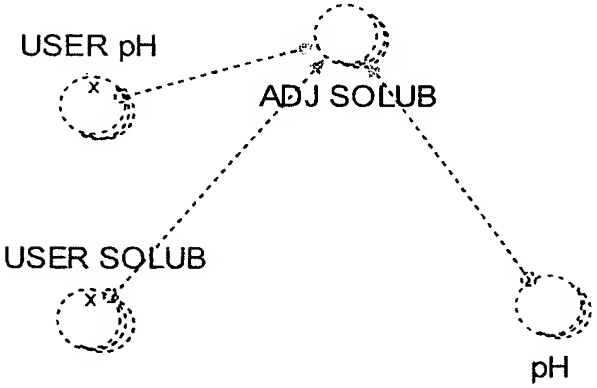

USER pH
ADJ SOLUB
USER SOLUB
pH

FIG. 33

Control Release Calculation

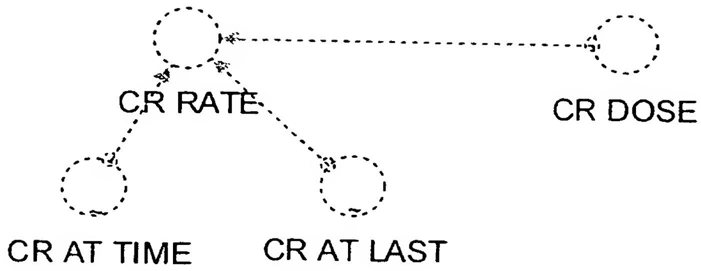

CR RATE
CR DOSE
CR AT TIME
CR AT LAST

FIG. 34

Concentration Calculation

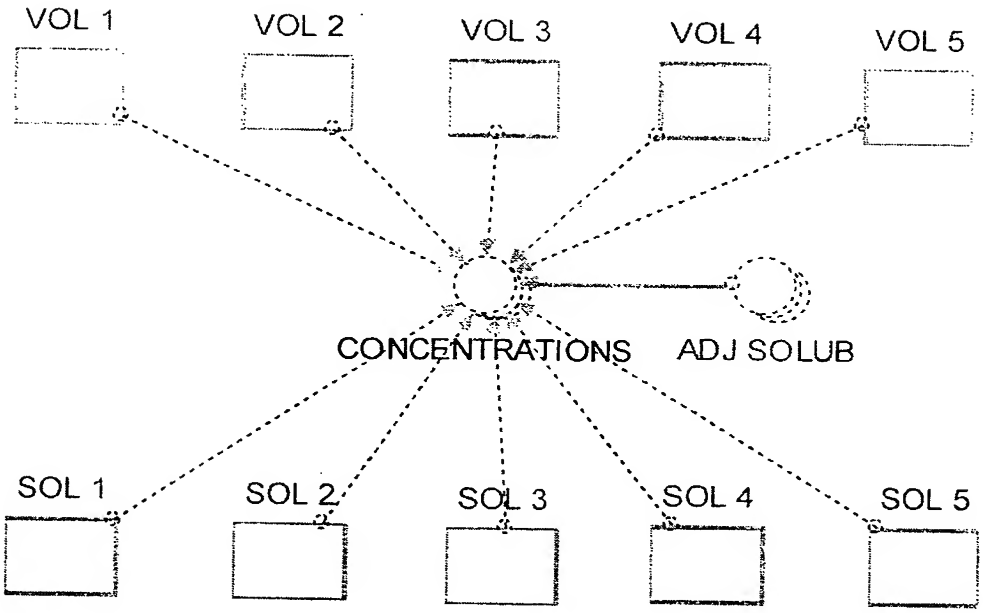

VOL 1
VOL 2
VOL 3
VOL 4
VOL 5
CONCENTRATIONS
ADJ SOLUB
SOL 1
SOL 2
SOL 3
SOL 4
SOL 5

FIG. 36
Output Calculations

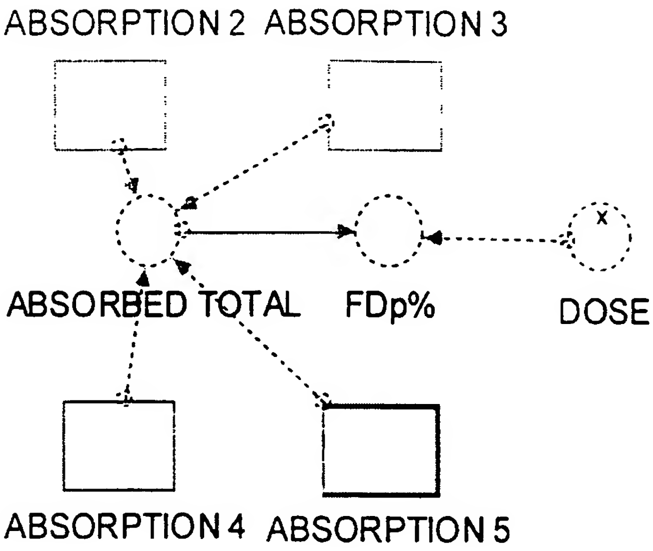

ABSORPTION 2
ABSORPTION 3
ABSORBED TOTAL
FDp%
X
DOSE
ABSORPTION 4
ABSORPTION 5

FIG. 37

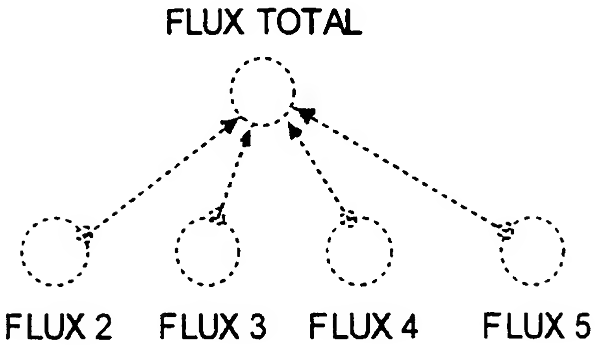

FLUX TOTAL
FLUX 2
FLUX 3
FLUX 4
FLUX 5

FIG. 35

Dissolution Calculation

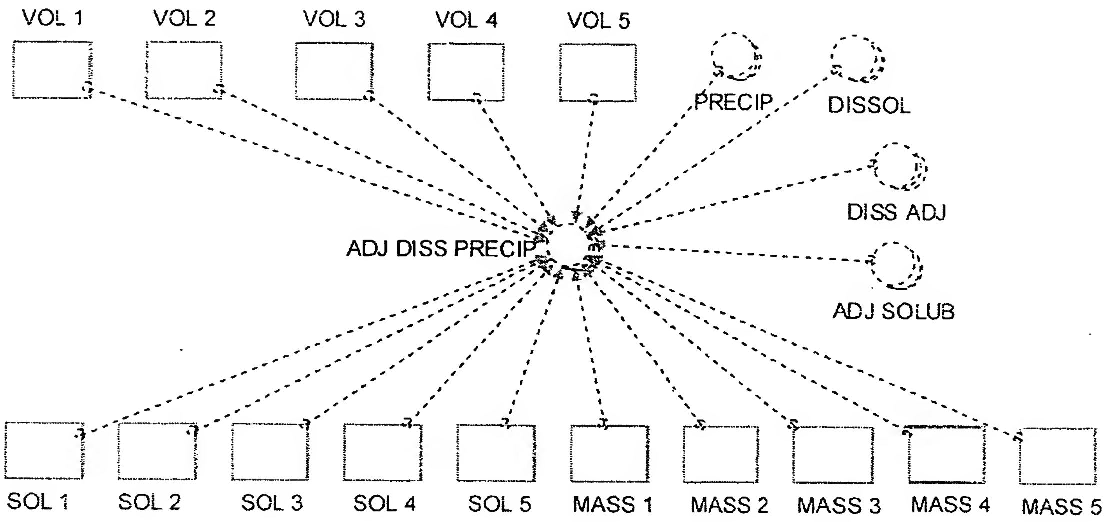

VOL 1
VOL 2
VOL 3
VOL 4
VOL 5
PRECIP
DISSOL
DISS ADJ
ADJ DISS PRECIP
ADJ SOLUB
SOL 1
SOL 2
SOL 3
SOL 4
SOL 5
MASS 1
MASS 2
MASS 3
MASS 4
MASS 5

FIG. 38

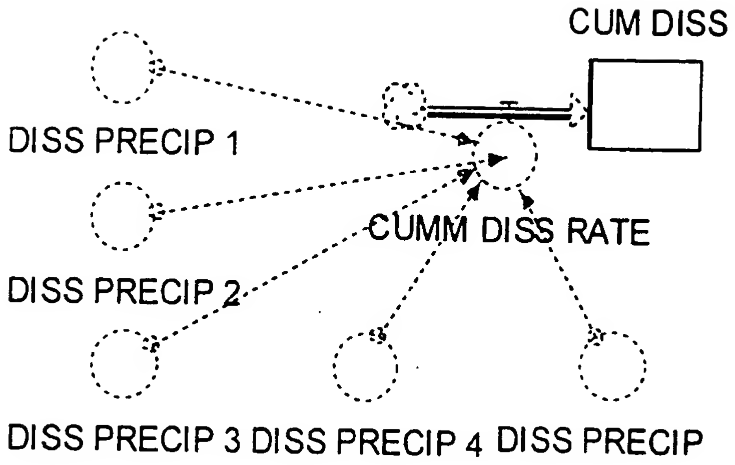

CUM DISS
DISS PRECIP 1
CUMM DISS RATE
DISS PRECIP 2
DISS PRECIP 3
DISS PRECIP 4
DISS PRECIP

FIG. 39

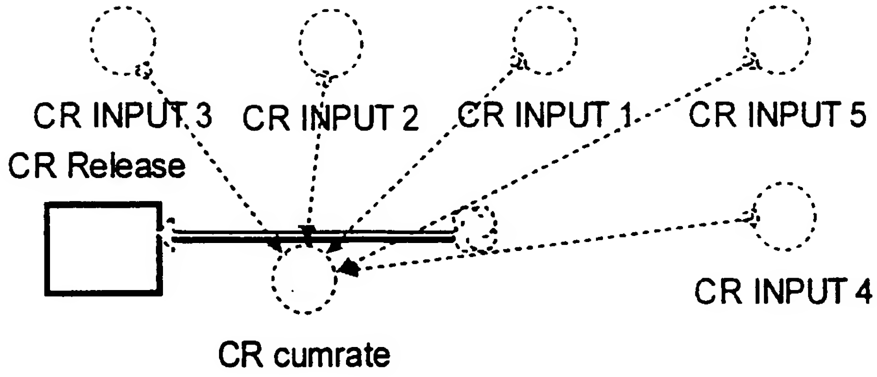

CR INPUT 3
CR INPUT 2
CR INPUT 1
CR INPUT 5
CR Release
CR INPUT 4
CR cumrate

FIG. 40

Physiological GI Tract Model

Database

- **GI Segment Compartments**
  - Fluid Absorption
  - Fluid Volume
  - Insoluble Mass
  - Soluble Mass
  - Soluble Mass Absorption
  - Dosage Form Mass

- **GI Segment Flow Regulators**
  - Fluid Absorption Rate
  - Fluid Volume Transit Rate
  - Insoluble Mass Transit Rate
  - Insoluble Mass Dissolution Rate
  - Soluble Mass Transit Rate
  - Soluble Mass Absorption Rate
  - Dosage Form Disintegration/Release Rate

- **GI Segment Converters**
  - Fluid Volume Absorption Rate Constant
  - GI Transit Rate Constant
  - Adjusted Dissolution Rate Constant
  - Dissolved Drug Concentration
  - Adjusted Surface Area
  - Adjusted Permeability

Rulebase

- GI Transit
- Dissolution
- Absorption
- Permeability Calculations
- Concentration Calculations
- Computational Error Corrections

FIG. 41

Physiological GI Tract Model

- **GI Segment Compartments & *Flow Regulators***
  - **Fluid Absorption**
    - *Fluid Absorption Rate*
  - **Fluid Volume**
    - *Fluid Volume Absorption Rate*
    - *Fluid Volume Transit Rate*
  - **Insoluble Mass**
    - *Insoluble Mass Transit Rate*
    - *Insoluble Mass Dissolution Rate*
  - **Soluble Mass**
    - *Insoluble Mass Dissolution Rate*
    - *Soluble Mass Transit Rate*
    - *Soluble Mass Absorption Rate*
  - **Soluble Mass Absorption**
    - *Soluble Mass Absorption Rate*

FIG. 42

Physiological GI Tract Model

- **GI Segments Flow Regulators & *Converters***
  - **Fluid Absorption Rate**
    - *Fluid Volume*
    - *Fluid Volume Absorption Rate Constant*
  - **Fluid Volume Transit Rate**
    - *Fluid Volume*
    - *Fluid Volume Transit Rate Constant*
  - **Insoluble Mass Transit Rate**
    - *Insoluble Mass*
    - *Insoluble Mass Transit Rate Constant*
  - **Insoluble Mass Dissolution Rate**
    - *Insoluble Mass*
    - *Dissolution Rate Constant*
  - **Soluble Mass Transit Rate**
    - *Soluble Mass*
    - *Soluble Mass Transit Rate Constant*
  - **Soluble Mass Absorption Rate (Flux)**
    - *Surface Area*
    - *Dissolved Mass Concentration*
    - *Permeability*

FIG. 43

Physiological GI Tract Model

- **Converters**
  - Permeability
    - Passive Absorption Adjustment Parameter
      - Efflux/Secretion Adjustment Parameter
      - Active Absorption Adjustment Parameter
      - Active or Carrier Mediated Absorptive Permeability
      - Km
      - Passive Permeability/Regional Correlation
        - Passive Permeability
        - Logic Function For Regional Correlation
          - Passive Permeability
          - Logic Function For Regional Correlation
      - Dissolved Mass Concentrations
  - Dissolved Mass Concentration
    - Fluid Volume
    - Solubility
      - pH
      - Solubility
  - Dissolution Rate Constant
    - Fluid Volume
    - Precipitation Rate Constant
    - Dissolution Rate Adjustment Parameter
    - Solubility
    - Insoluble Mass
    - Soluble Mass
  - Surface Area
    - Surface Area Adjustment Parameter
    - Transport Mechanism
  - Transit Rate
    - Transit Time Adjustment Parameter
    - User Adjusted Transit Time
  - Fluid Volume Absorption Rate Constant
    - Fluid Volume Adjustment Parameter

FIG. 44

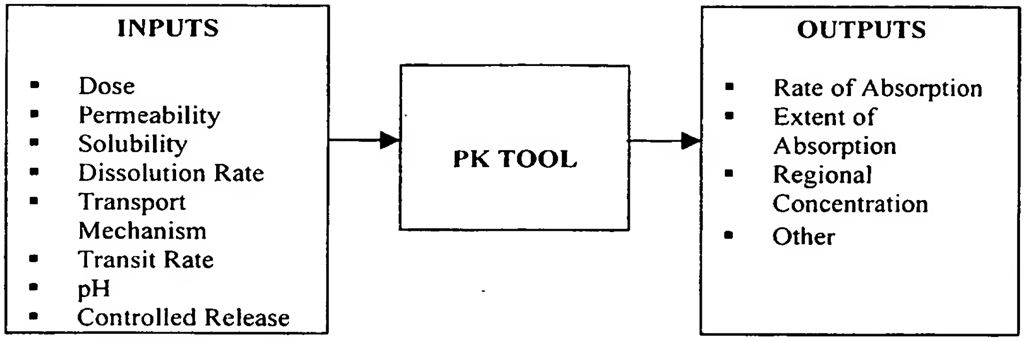

INPUTS
Dose
Permeability
Solubility
Dissolution Rate
Transport Mechanism
Transit Rate
pH
Controlled Release
PK TOOL
OUTPUTS
Rate of Absorption
Extent of Absorption
Regional Concentration
Other

FIG. 45

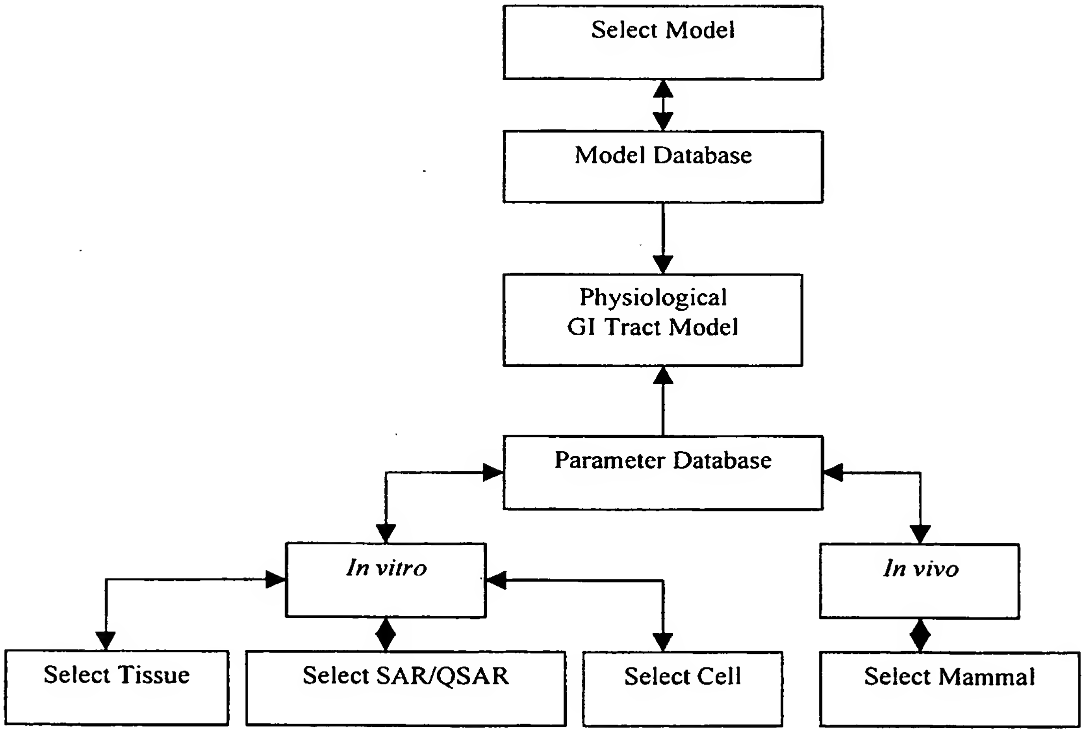

Select Model
Model Database
Physiological GI Tract Model
Parameter Database
In vitro
In vivo
Select Tissue
Select SAR/QSAR
Select Cell
Select Mammal

FIG. 46

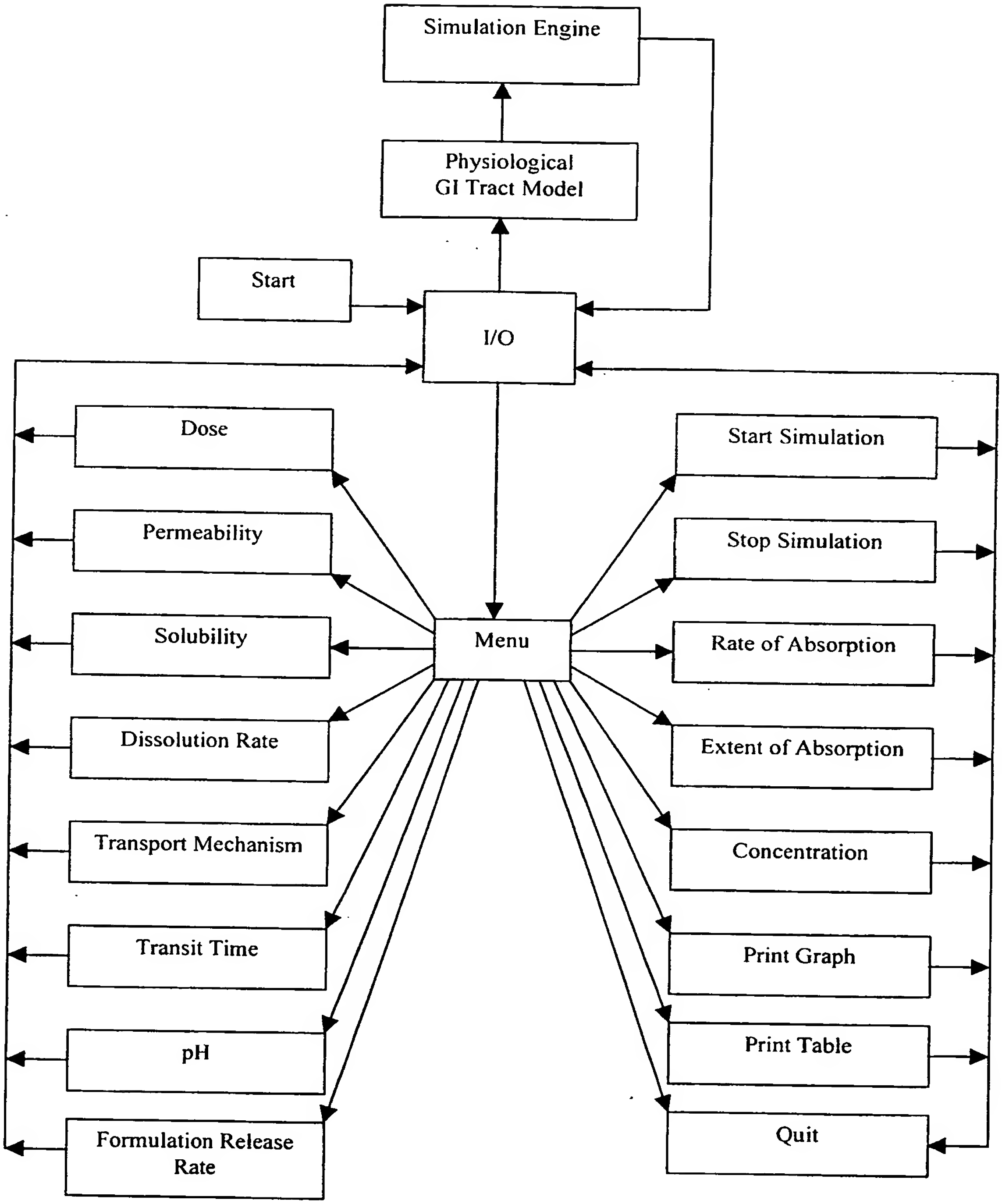

Simulation Engine
Physiological GI Tract Model
Start
I/O
Dose
Permeability
Solubility
Dissolution Rate
Transport Mechanism
Transit Time
pH
Formulation Release Rate
Menu
Start Simulation
Stop Simulation
Rate of Absorption
Extent of Absorption
Concentration
Print Graph
Print Table
Quit

FIG. 47

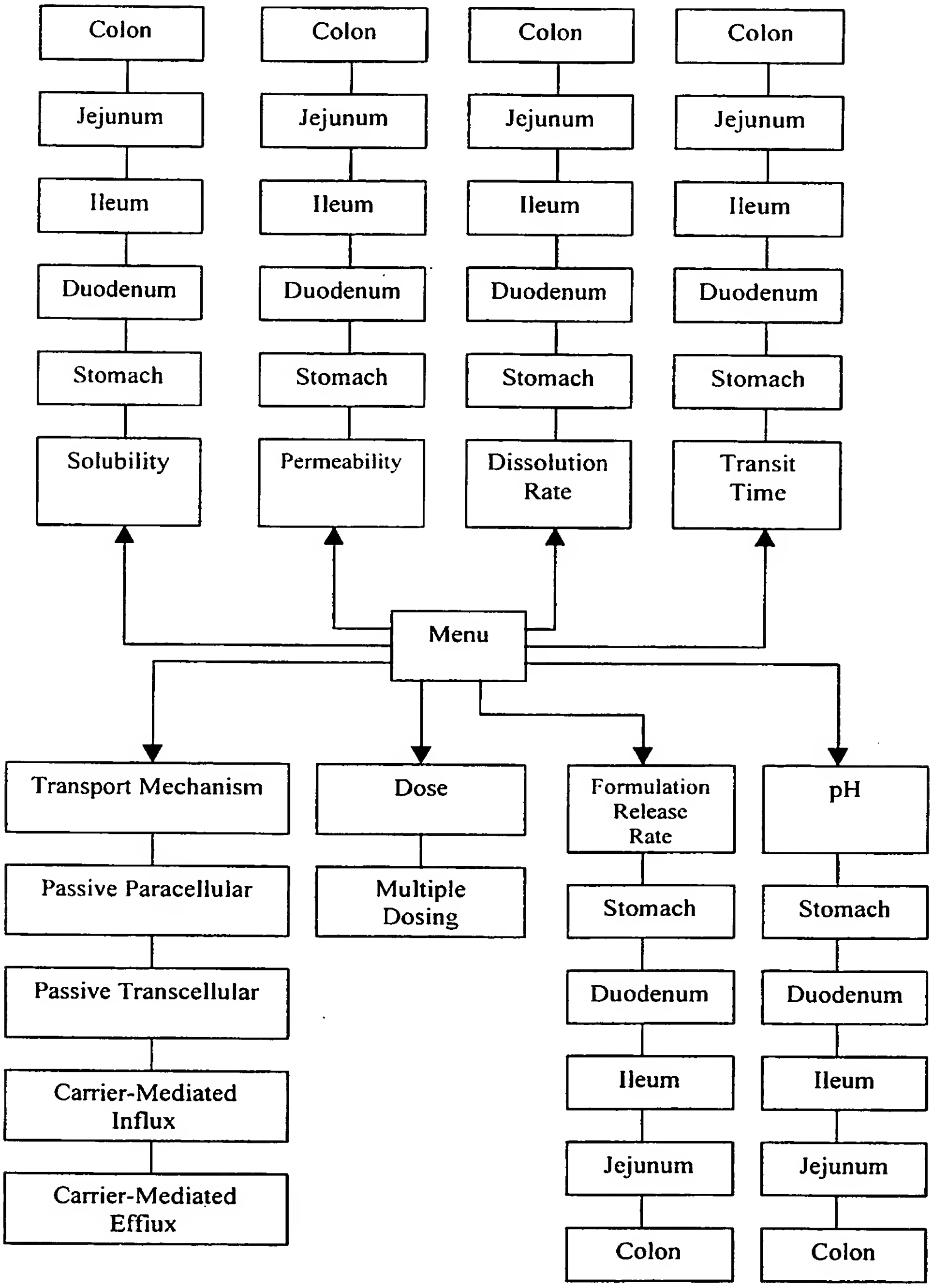

Colon
Jejunum
Ileum
Duodenum
Stomach
Solubility
Colon
Jejunum
Ileum
Duodenum
Stomach
Permeability
Colon
Jejunum
Ileum
Duodenum
Stomach
Dissolution Rate
Colon
Jejunum
Ileum
Duodenum
Stomach
Transit Time
Menu
Transport Mechanism
Passive Paracellular
Passive Transcellular
Carrier-Mediated Influx
Carrier-Mediated Efflux
Dose
Multiple Dosing
Formulation Release Rate
Stomach
Duodenum
Ileum
Jejunum
Colon
pH
Stomach
Duodenum
Ileum
Jejunum
Colon

FIG. 48

Figure F1.

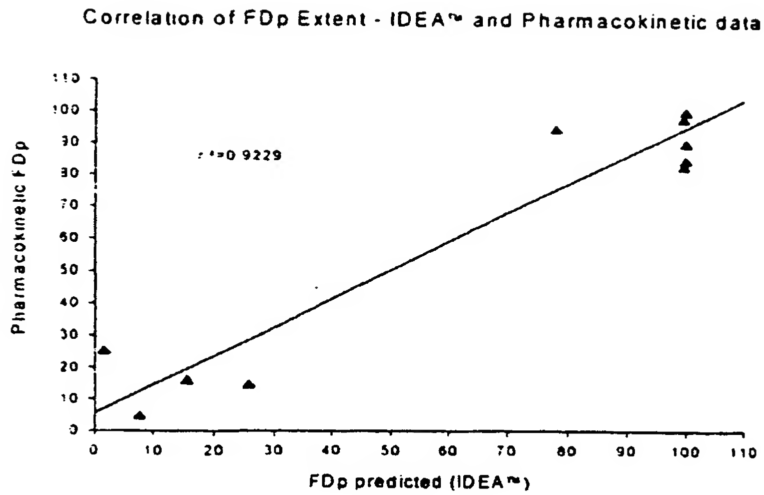

Correlation of FDp Extent - IDEA™ and Pharmacokinetic data

FIG. 49

Figure 2:

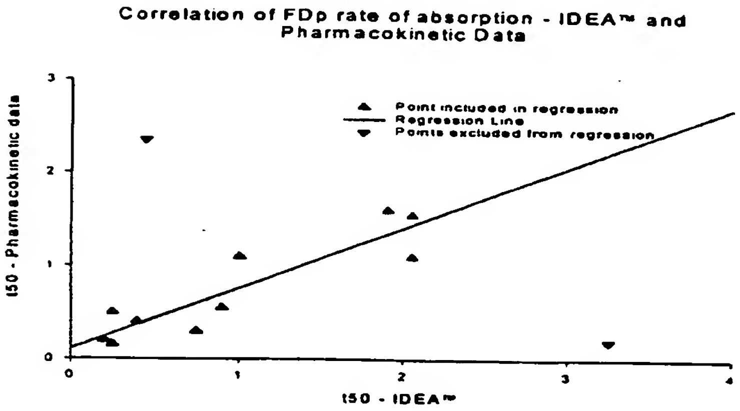

Correlation of FDp rate of absorption - IDEA™ and Pharmacokinetic Data

FIG. 50

Figure F3.

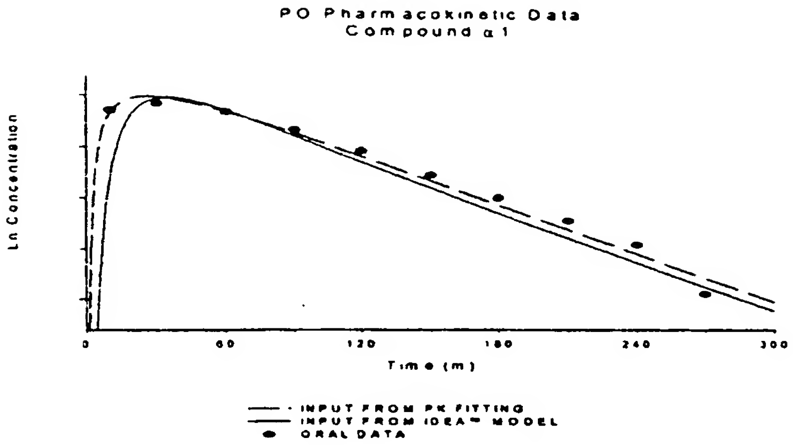


FIG. 51

Figure F4:

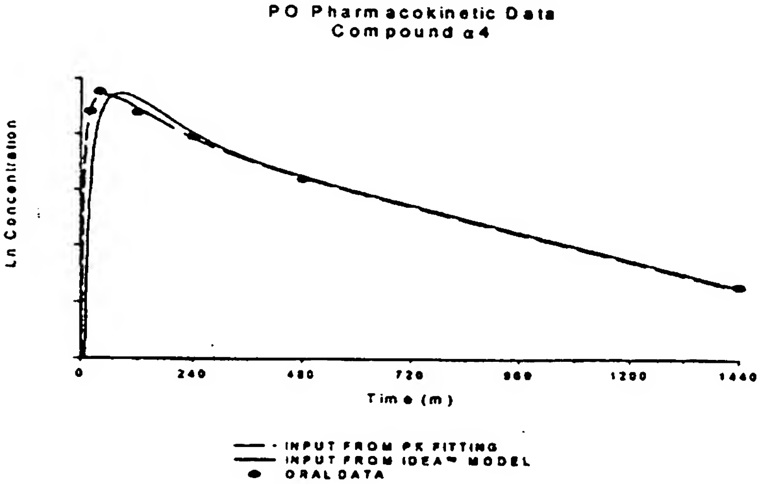


FIG. 52

Figure F5:

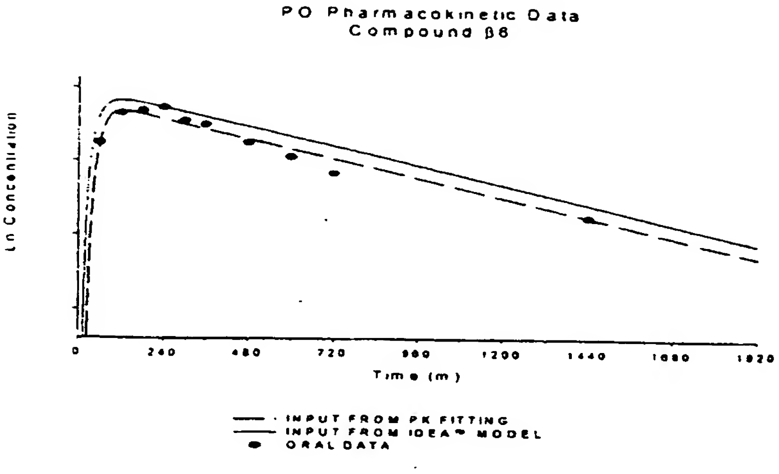


FIG. 53

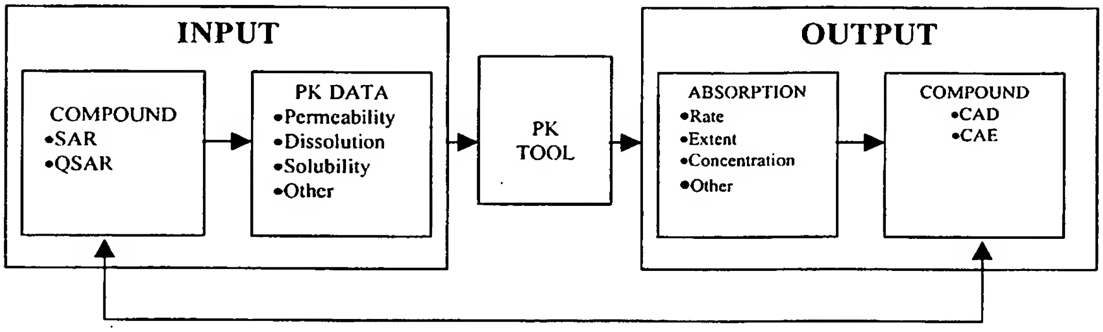
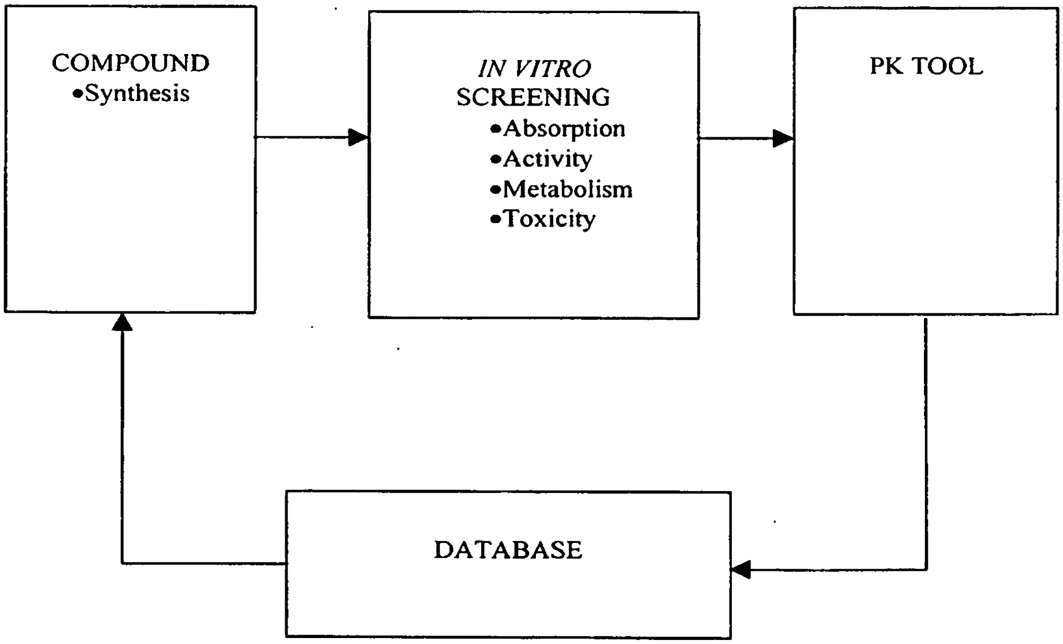

INPUT
COMPOUND
•SAR
•QSAR
PK DATA
•Permeability
•Dissolution
•Solubility
•Other
PK
TOOL
OUTPUT
ABSORPTION
•Rate
•Extent
•Concentration
•Other
COMPOUND
•CAD
•CAE

FIG. 54


COMPOUND
•Synthesis
IN VITRO
SCREENING
•Absorption
•Activity
•Metabolism
•Toxicity
PK TOOL
DATABASE

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
23 March 2000 (23.03.2000)

PCT

(10) International Publication Number
WO 00/15178 A3

(51) International Patent Classification⁷: C12Q 1/00

(21) International Application Number: PCT/US99/21001

(22) International Filing Date: 14 September 1999 (14.09.1999)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/100,224	14 September 1998 (14.09.1998)	US
60/100,290	14 September 1998 (14.09.1998)	US
60/109,232	18 November 1998 (18.11.1998)	US
60/109,234	18 November 1998 (18.11.1998)	US
09/320,069	26 May 1999 (26.05.1999)	US
09/320,270	26 May 1999 (26.05.1999)	US
09/320,371	26 May 1999 (26.05.1999)	US
09/320,372	26 May 1999 (26.05.1999)	US
09/320,544	26 May 1999 (26.05.1999)	US
09/320,545	26 May 1999 (26.05.1999)	US

(71) Applicant *(for all designated States except US)*: **NAVICYTE, INC.** [US/US]; 9880 Campus Point Drive, San Diego, CA 92121 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **GRASS, George, M.** [US/US]; 1506 Juniper Mountain Road, P.O. Box 1242, Tahoe City, CA 96145 (US). **LEESMAN, Glen, D.** [US/US]; 186 Nighthawk Lane, Hamilton, MT 59840-9307 (US). **NORRIS, Daniel, A.** [US/US]; 3145 Cowley Way #130, San Diego, CA 92117 (US). **SINKO, Patrick, J.** [US/US]; 2 Country Place, Lebanon, NJ 08833 (US). **WEHRLI, John, E.** [US/US]; 4225 Corte de la Siena, San Diego, CA 92130 (US).

(74) Agents: POSTNER, Marya, A.; Cooley Godward LLP, 3000 El Camino Real, Five Palo Alto Square, Palo Alto, CA 94306-2155 et al. (US).

(81) Designated States *(national)*: AU, CA, JP, US.

(84) Designated States *(regional)*: European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

(88) Date of publication of the international search report: 26 July 2001

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: COMPUTER IMPLEMENTED PHARMACOKINETICS METHOD AND PROGRAM

(57) Abstract: The present invention relates to a pharmacokinetic-based design and selection tool (PK tool) and methods for predicting absoption of an administered compound of interest. The methods utilize the tools, and optionally a separately operable component or subsystem thereof. The PK tool includes as computer-readable components: (1) input/output system; (2) physiologic-based simulation model of one or more segments of a mammalian system of interest having one or more physiological barriers to absorption that is based on the selected route of administration; and (3) simulation engine having a differential equation solver. The invention also provides methods for optimizing as well as enabling minimal input requirements a physiologic-based simulation model for predicting *in vivo* absorption, and optionally one or more additional properties, from either *in vitro* or *in vivo* data. The PK tool of the invention may be provided as a computer system, as an article of manufacture in the form of a computer-readable medium, or a computer program product and the like. Subsystems and individual components of the PK tool also can be utilized and adapted in a variety of disparate applications for predicting the fate of an administered compound. The PK tool and methods of the invention can be used to screen and design compound libraries, select and design drugs, as well as predict drug efficacy in mammals from *in vitro* and/or *in vivo* data of one or more compounds of interest. The PK tool and methods of the invention also find use in selecting, designing, and preparing drug compounds, and multi-compound drugs and drug formulations (i.e., drug delivery system) for preparation of medicaments for use in treating mammalian disorders.

WO 00/15178 A3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/21001

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :C12Q 1/00
US CL :702/19
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 702/19

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Remington's Pharmaceutical Sciences

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Database Derwent, Accession No: 1995-263280, CARELL et al, 'Biologically active molecule combinatorial library production - by reacting core molecule with tool molecules, especially for preparation of new xanthene derivative serine protease inhibitors', abstract, WO 95/19359 A, 20 July 1995, see entire abstract.	1-199
Y	US 5,789,160 A (EATON et al) 04 August 1998, see the entire document, especially the West search report last paragraph attached to issued patent, note that Eaton et al teach among the endpoint of library screening in the SELEX system includes pharmacokinetics, bioavailability and toxicity profiles.	1-199

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search 19 DECEMBER 1999	Date of mailing of the international search report 01 NOV 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer JOSEPH W. RICIGLIANO Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/21001

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,770,384 A (ANDROPHY et al) 23 June 1998, see the entire document, especially the attached WEST data base search, note the reference teaches screening libraries of test compounds for pharmacokinetic parameters including bioavailability.	1-199
Y	HARVEY, S. C. Remington's Pharmaceutical Sciences. Easton, Pennsylvania: Mack Publishing Co., 1990, Chapter 35, 'Drug Absorption, Action and Disposition', pages 697-724, and Chapter 36, 'Basic Pharmacokinetics', pages 725-745, see entire document.	1-199
Y	GEX-FABRY et al. Pharmacokinetics of Drugs. Berlin: Springer-Verlag. 1994, chapter entitled, 'Considerations on data analysis using computer methods and currently available software for personal computers', pages 507-527, see entire document.	1-199
Y	Database Dialog, INSPEC Abstract Number: C82024056, HOLFORD, N.H.G. 'DRUGMODEL (pharmacokinetic modelling),' abstract, Proceedings of the fifth annual symposium on computer applications in medicinal care.' New York: IEEE, 1981, pages 603-606, see entire abstract.	1-199

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/21001

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest
☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/21001

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

WEST: USPAT and DERWENT FILES, DIALOG, NCBI and NLM sand NIH web sites.
Search terms included: library, combinatorial, pharmacokinetics, pharmacodynamics, gastrointestinal, absorption, bioavailability, compartments, Prophet, Drugmodel, Stella, PCNONLIN, computer, microcomputer. LANGRAN

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claims 1-53, 80-121 and 137-199, drawn to computer implemented methods/systems and computer implemented article of manufacturer and products including computer systems configured to for pharmacokinetic analysis.
Group II, claims 54-79, drawn to methods of optimizing pharmacokinetics parameters.
Group III, claims 122-126, drawn to computer subsystems for modeling the GI tract and absorption.
Group IV, claims 127-130, drawn to databases for simulating compound absorption in a mammal.
Group V, claims 131-136, drawn to computer implemented model of the GI tract.

The inventions listed as Groups I-V do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:
The invention lack unit as each group of claims is drawn to methods or computer implemented products which have different steps and different requirements. Moreover, as computer implemented pharmacokinetics modeling is known in the art the claimed inventions do not share a special technical feature.

This application contains claims directed to more than one species of the generic invention within groups I-VI. These species are deemed to lack Unity of Invention because they are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for more than one species to be searched, the appropriate additional search fees must be paid. The species are as follows:
i, a mammalian system of interest.
ii, a parameter for which the differential equations are calculated.
The species listed above do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons: Each mammalian represents a species of organ system (compartment) for which pharmacokinetics parameters will be used. This is recognized by the art and as such the recitation of different systems does not correspond to a special technical feature but rather simply a technical feature. Similarly the measurement of different parameters which are recognized in the art and the calculation of differential based equations for their presence and rates of transfer is also known in the art and hence does not constitute a special technical feature.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

CORRECTED VERSION

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
23 March 2000 (23.03.2000)

PCT

(10) International Publication Number
WO 00/15178 A3

(51) **International Patent Classification^7:** C12Q 1/00

(21) **International Application Number:** PCT/US99/21001

(22) **International Filing Date:**
14 September 1999 (14.09.1999)

(25) **Filing Language:** English

(26) **Publication Language:** English

(30) **Priority Data:**

60/100,224	14 September 1998 (14.09.1998)	US
60/100,290	14 September 1998 (14.09.1998)	US
60/109,232	18 November 1998 (18.11.1998)	US
60/109,234	18 November 1998 (18.11.1998)	US
09/320,069	26 May 1999 (26.05.1999)	US
09/320,270	26 May 1999 (26.05.1999)	US
09/320,371	26 May 1999 (26.05.1999)	US
09/320,372	26 May 1999 (26.05.1999)	US
09/320,544	26 May 1999 (26.05.1999)	US
09/320,545	26 May 1999 (26.05.1999)	US

(71) **Applicant** *(for all designated States except US)*: **NAVICYTE, INC.** [US/US]; 9880 Campus Point Drive, San Diego, CA 92121 (US).

(72) **Inventors; and**
(75) **Inventors/Applicants** *(for US only)*: **GRASS, George, M.** [US/US]; 1506 Juniper Mountain Road, P.O. Box 1242, Tahoe City, CA 96145 (US). **LEESMAN, Glen, D.** [US/US]; 186 Nighthawk Lane, Hamilton, MT 59840-9307 (US). **NORRIS, Daniel, A.** [US/US]; 3145 Cowley Way #130, San Diego, CA 92117 (US). **SINKO, Patrick, J.** [US/US]; 2 Country Place, Lebanon, NJ 08833 (US). **WEHRLI, John, E.** [US/US]; 4225 Corte de la Siena, San Diego, CA 92130 (US).

(74) **Agents: POSTNER, Marya, A.**; Cooley Godward LLP, 3000 El Camino Real, Five Palo Alto Square, Palo Alto, CA 94306-2155 et al. (US).

(81) **Designated States** *(national)*: AU, CA, JP, US.

(84) **Designated States** *(regional)*: European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

(88) **Date of publication of the international search report:**
26 July 2001

(48) **Date of publication of this corrected version:**
4 October 2001

(15) **Information about Correction:**
see PCT Gazette No. 40/2001 of 4 October 2001, Section II

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: COMPUTER IMPLEMENTED PHARMACOKINETICS METHOD AND PROGRAM

(57) **Abstract:** The present invention relates to a pharmacokinetic-based design and selection tool (PK tool) and methods for predicting absoption of an administered compound of interest. The methods utilize the tools, and optionally a separately operable component or subsystem thereof. The PK tool includes as computer-readable components: (1) input/output system; (2) physiologic-based simulation model of one or more segments of a mammalian system of interest having one or more physiological barriers to absorption that is based on the selected route of administration; and (3) simulation engine having a differential equation solver. The invention also provides methods for optimizing as well as enabling minimal input requirements a physiologic-based simulation model for predicting *in vivo* absorption, and optionally one or more additional properties, from either *in vitro* or *in vivo* data. The PK tool of the invention may be provided as a computer system, as an article of manufacture in the form of a computer-readable medium, or a computer program product and the like. Subsystems and individual components of the PK tool also can be utilized and adapted in a variety of disparate applications for predicting the fate of an administered compound. The PK tool and methods of the invention can be used to screen and design compound libraries, select and design drugs, as well as predict drug efficacy in mammals from *in vitro* and/or *in vivo* data of one or more compounds of interest. The PK tool and methods of the invention also find use in selecting, designing, and preparing drug compounds, and multi-compound drugs and drug formulations (i.e., drug delivery system) for preparation of medicaments for use in treating mammalian disorders.

WO 00/15178 A3

Computer Implemented
Pharmacokinetics Method and Program

INTRODUCTION

Technical Field

The present invention relates to computer-implemented pharmacokinetic simulation models and drug design.

Background

A. Pharmacokinetic Modeling

Pharmacodynamics refers to the study of fundamental or molecular interactions between drug and body constituents, which through a subsequent series of events results in a pharmacological response. For most drugs the magnitude of a pharmacological effect depends on time-dependent concentration of drug at the site of action (e.g., target receptor-ligand/drug interaction). Factors that influence rates of delivery and disappearance of drug to or from the site of action over time include absorption, distribution, metabolism, and elimination. The study of factors that influence how drug concentration varies with time is the subject of pharmacokinetics.

In nearly all cases the site of drug action is located on the other side of a membrane from the site of drug administration. For example, an orally administered drug must be absorbed across a membrane barrier at some point or points along the gastrointestinal (GI) tract. Once the drug is absorbed, and thus passes a membrane barrier of the GI tract, it is transported through the portal vein to the liver and then eventually into systemic circulation (i.e., blood and lymph) for delivery to other body parts and tissues by blood flow. Thus how well a drug crosses membranes is of key importance in assessing the rate and extent of absorption and distribution of the drug throughout different body compartments and tissues. In essence, if an otherwise highly potent drug is administered extravascularly (e.g., oral) but is poorly absorbed (e.g., GI tract), a majority of the drug will be excreted or eliminated and thus cannot be distributed to the site of action.

The principle routes by which drugs disappear from the body are by elimination of unchanged drug or by metabolism of the drug to a pharmacologically active or inactive form(s) (i.e., metabolites). The metabolites in turn may be subject to further elimination or metabolism. Elimination of drugs occurs mainly via renal mechanisms into the urine and to some extent via mixing with bile salts for solubilization followed by excretion through the GI tract, exhaled through the lungs, or secreted through sweat or salivary glands etc. Metabolism for most drugs occurs primarily in the liver.

Each step of drug absorption, distribution, metabolism, and elimination can be described mathematically as a rate process. Most of these biochemical processes involve first order or pseudo-first order rate processes. In other words, the rate of reaction is proportional to drug concentration. For instance, pharmacokinetic data analysis is based on empirical observations after administering a known dose of drug and fitting of the data by either descriptive equations or mathematical (compartmental) models. This permits summarization of the experimental measures (plasma/blood level-time profile) and prediction under many experimental conditions. For example after rapid intravenous administration, drug levels often decline mono-exponentially (first-order elimination) with respect to time as described in Equation 1, where Cp(t) is drug concentration as a function of time, Cp(0) is initial drug concentration, and k is the associated rate constant that represents a combination of all factors that influence the drug decay process (e.g., absorption, distribution, metabolism, elimination).

$$Cp(t) = Cp(0)e^{-kt} \qquad \text{(Eq. 1)}$$

This example assumes the body is a single "well-mixed" compartment into which drug is administered and from which it also is eliminated (one-compartment open model). If equilibrium between drug in a central (blood) compartment and a (peripheral) tissue compartment(s) is not rapid, then more complex profiles (multi-exponential) and models (two- and three-compartment) are used. Mathematically, these "multi-compartment" models are described as the sum of equations, such as the sum of rate processes each calculated according to Equation 1 (i.e., linear pharmacokinetics).

Experimentally, Equation 1 is applied by first collecting time-concentration data from a subject that has been given a particular dose of a drug followed by plotting the data points on a logarithmic graph of time versus drug concentration to generate one type of time-concentration curve. The slope (k) and the y-intercept (C0) of the plotted "best-fit" curve is obtained and subsequently incorporated into Equation 1 (or sum of equations) to describe the drug's time course for additional subjects and dosing regimes.

When drug concentration throughout the body or a particular location is very high, saturation or nonlinear pharmacokinetics may be applicable. In this situation the capacity of a biochemical and/or physiological process to reduce drug concentration is saturated. Conventional Michaelis-Menten type equations are employed to describe the nonlinear nature of the system, which involve mixtures of zero-order (i.e., saturation:concentration independent) and first-order (i.e., non-saturation:concentration dependent) kinetics. Experimentally, data collection and plotting are similar to that of standard compartment models, with a notable exception being that the data curves are nonlinear. Using a time versus concentration graph to illustrate this point, at very high drug concentration the data line is linear because the drug is being eliminated at a maximal constant rate (i.e., zero-order process). The data line then begins to curve in an asymptotic fashion with time until the drug concentration drops to a point where the rate process becomes proportional to drug concentration (i.e., first-order process). For many drugs, nonlinear pharmacokinetics applies to events such as dissolution of the therapeutic ingredient from a drug formulation, as well as metabolism and elimination. Nonlinear pharmacokinetics also can be applied to toxicological events related to threshold dosing.

Classical one, two and three compartment models used in pharmacokinetics require *in vivo* blood data to describe time-concentration effects related to the drug decay process, i.e., blood data is relied on to provide values for equation parameters. For instance, while a model may work to describe the decay process for one drug, it is likely to work poorly for others unless blood profile data and associated rate process limitations are generated for each drug in question. Thus, such models are very poor for predicting the *in vivo* fate of diverse drug sets in the absence of blood data and the like derived from animal and/or human testing.

In contrast to the standard compartment models, physiological-based pharmacokinetic models are designed to integrate basic physiology and anatomy with drug distribution and disposition. Although a compartment approach also is used for physiological models, the compartments correspond to anatomic entities such as the GI tract, liver, lung etc., which are connected by blood flow. Physiological modeling also differs from standard compartment modeling in that a large body of physiological and physicochemical data usually is employed that is not drug-specific. However, as with standard compartment models the conventional physiological models lump rate processes together. Also, conventional physiological models typically fail to incorporate individual kinetic, mechanistic and physiological processes that control drug distribution and disposition in a particular anatomical entity, even though multiple rate processes are represented *in vivo*. Physiological models that ignore these and other important model parameters contain an underlying bias resulting in poor correlation and predictability across diverse data sets. Such deficiencies inevitably result in unacceptable levels of error when the model is used to describe or predict drug fate in animals or humans. The problem is amplified when the models are employed to extrapolate animal data to humans, and worse, when *in vitro* data is relied on for prediction in animals or humans.

For instance, the process of drug reaching the systemic circulation for most orally administered drugs can be broken down into two general steps: dissolution and absorption. Since endocytotic processes in the GI tract typically are not of high enough capacity to deliver therapeutic amounts of most drugs, the drugs must be solubilized prior to absorption. The process of dissolution is fairly well understood. However, the absorption process is treated as a "black box." Indeed, although bioavailability data is widely available for many drugs in multiple animal species and in humans, *in vitro* and or *in vivo* data generated from animal, tissue or cell culture permeability experiments cannot allow a direct prediction of drug absorption in humans, although such correlations are commonly used.

B. Computer Systems and Pharmacokinetic Modeling

Computers have been used in pharmacokinetics to bring about easy solutions to complex pharmacokinetic equations and modeling of pharmacokinetic processes. Other computer applications in pharmacokinetics include development of experimental study designs, statistical data treatment, data manipulation, graphical representation of data, projection of drug action, as well as preparation of written reports or documents.

Since pharmacokinetic models are described by systems of differential equations, virtually all computer systems and programming languages that enable development and implementation of mathematical models have been utilized to construct and run them. Graphics-oriented model development computer programs, due to their simplicity and ease of use, are typically used for designing multi-compartment linear and non-linear pharmacokinetic models. In essence, they allow a user to interactively draw compartments and then link and modify them with other iconic elements to develop integrated flow pathways using pre-defined symbols. The user assigns certain parameters and equations relating the parameters to the compartments and flow pathways, and then the model development program generates the differential equations and interpretable code to reflect the integrated system in a computer-readable format. The resulting model, when provided with input values for parameters corresponding to the underlying equations of the model, such as drug dose and the like can then be used to simulate the system under investigation.

While tools to develop and implement pharmacokinetic models exist and the scientific literature is replete with examples, pharmacokinetic models and computer systems developed to date have not permitted sufficient predictability of the pharmacokinetic fate of extravascularly administered drugs in a mammal from *in vitro* cell, tissue or compound structure-activity relationship (SAR/QSAR) data. A similar problem exists when attempting to predict absorption of a compound in one mammal (e.g., human) from data derived from a second mammal (e.g., dog). For example, existing pharmacokinetic models of oral absorption use several different approaches to predict oral absorption and fraction dose absorbed (Amidon *et al., Pharm. Res., (1988)* 5:651-654; Chiou, W.L., *Int. J. Clin. Pharmacol. Ther., (1994)* 32:474-482; Chiou, W.L., *Biopharm.Drug Dispos., (1995)* 16:71-75; Dressman *et al., J. Pharm. Sci.,* (1985) 74:588-589; Lennernas *et al., J Pharm. Pharmacol.,* (1997) 49:682-686; Levet-Trafit *et al., Life Sciences., (1996)* 58:PL359-63; Sinko *et al., Pharm. Res., (1991)* 8:979-988; and Soria *et al.,. Biopharm. Drug Dispos., (1996)* 17:817-818). Unfortunately, these models are flawed as they make mathematical assumptions that limit prediction to particular compounds, and the correlation function is sigmoidal in shape (i.e., high/steep slope). Therefore the predictive power of such models for compounds outside a relatively small group is very limited. This is particularly true for collections of compounds possessing variable ranges of dosing requirements and of permeability, solubility, dissolution rates and transport mechanism properties. Other drawbacks include use of drug-specific parameters and values in pharmacokinetic models from the outset of model development, which essentially limits the models to drug-specific predictions. These and other deficiencies also impair generation of rules that universally apply to drug disposition in a complex physiological system such as the GI tract.

Extravascular administration of drugs is the preferred route for physicians, patients, and drug developers alike due to lower product price, increased patient compliance, ease of administration. Current assessment of the bioavailability of extravascularly administered drugs and lead drug compounds, as well as bioavailability of intravascularly administered compounds relative to specialized barriers to absorption such as the blood brain barrier, is limited in large part to animal and human testing. The economic and medical consequences of problems with drug absorption and variable bioavailability are immense. Failing to identify promising or potentially problematic drug candidates during the discovery and pre-clinical stages of drug development is one of the most significant consequences of problems with drug bioavailability. Accordingly, there is a need to develop a comprehensive, physiologically-based pharmacokinetic model and computer system capable of predicting drug bioavailability and variability in humans that utilizes relatively straightforward input parameters. Furthermore, considering the urgent need to provide the medical community with new therapeutic alternatives and the current use of high throughput drug screening for selecting lead drug candidates, a comprehensive biopharmaceutical computer-based tool that employs a modeling approach for predicting bioavailability of compounds and compound formulations is needed.

Relevant Literature

Various publications review gastrointestinal anatomy and physiology including motility, secretion, absorption, and digestion, as well as gastrointestinal pharmacology and physiology in gastrointestinal diseased individuals (See, e.g., L. Johnson ed., *Physiology of the Gastrointestinal Tract,* Second edition, Vol. 2, Ravind Press (1987); Kutchai, *Gastrointestinal System, Part IV., Principles of Physiology,* Mosby Press (1996); and Sleisenger, *Gastrointestinal Disease,* 3rd edition, Saunders (1983)). Sharget *et al.* (*Physiological Factors Related to Drug Absorption*, Applied Biopharmaceutics and Pharmacokinetics (1993)) review pharmacokinetics and compartment modeling. Various pharmacokinetic models of oral drug absorption are disclosed in Grass, G. (*Advanced Drug Delivery Reviews* (1997) 23:199-219); Amidon et al., (*Pharm. Res.* (1988) 5:651-654); Chiou, W.L., (*Int. J. Clin. Pharmacol. Ther.*, (1994) 32:474-482); Chiou, W.L., (*Biopharm. Drug Dispos.*, (1995) 16:71-75); Dressman et al., (*J. Pharm. Sci.*, (1985) 74:588-589); Lennernas et al., (*J Pharm. Pharmacol.*, (1997) 49:682-686); Levet-Trafit et al., (*Life Sciences.*, (1996) 58:PL359-63); Sinko et al., (*Pharm. Res.*, (1991) 8:979-988); and Soria et al.,. (*Biopharm. Drug Dispos.*, (1996) 17:817-818)).

SUMMARY OF THE INVENTION

The present invention relates to a pharmacokinetic-based design and selection tool (PK tool) and methods for predicting absorption of a compound in a mammalian system of interest. The methods utilize the tool, and optionally a separately operable component or subsystem thereof.

The PK tool comprises as computer-readable components: (1) input/output system; (2) physiologic-based simulation model of one or more segments of a mammalian system of interest having one or more physiological barriers to absorption that is based on the selected route of administration; and (3) simulation engine having a differential equation solver, and optionally, a control statement module. The physiologic-based simulation model of the PK tool of the invention is a multi-compartment mathematical model comprising as operably linked components: (i) differential equations for one or more of fluid transit, fluid absorption, mass transit, mass dissolution, mass solubility, and mass absorption for one or more segments of the mammalian system of interest; and (ii) initial parameter values for the differential equations corresponding to physiological parameters and selectively optimized adjustment parameters, and optionally regional correlation parameters, for one or more segments of the mammalian system of interest; and, optionally, (iii) control statement rules for one or more of transit, absorption, permeability, solubility, dissolution, concentration, and mathematical error correction for one or more segments of the mammalian system of interest.

The computer-readable input/output system, physiologic-based simulation model, and simulation engine of the PK tool are capable of working together to carrying out the steps of: (1) receiving through the input/output system data comprising dose, permeability and solubility data of a compound of interest for one or more segments of the mammalian system of interest; and (2) applying the physiologic-based simulation model and simulation engine to generate an absorption profile for the compound characterized by one or more of concentration, rate of absorption, and extent of absorption relative to a selected sampling site that is across a physiological barrier for one or more segments of the mammalian system of interest.

The present invention also provides a database for utilization in the PK tool and method of the invention. The database includes one or more physiologic-based simulation models of the invention. Additional databases are provided for simulation model parameters, and may be integrated or separate from a database having a simulation model of the invention. The database(s) includes one or more of (i) differential equations for one or more of fluid transit, fluid absorption, mass transit, mass dissolution, mass solubility, and mass absorption for one or more segments of the mammalian system of interest; (ii) initial parameter values for the differential equations corresponding to physiological parameters and selectively optimized adjustment parameters, and optionally regional correlation parameters, for one or more segments of the mammalian system of interest; and (iii) control statement rules for one or more of transit, absorption, permeability, solubility, dissolution, concentration, and mathematical error correction for one or more segments of the mammalian system of interest. The database(s) have a compartment-flow data structure that is portable into and readable by a simulation engine for calculating time-dependent rate of absorption, extent of absorption, and concentration of a compound at a sampling site across a physiological barrier of one or more segments of the mammalian system of interest.

The invention also includes a method for selectively optimizing a pharmacokinetic-based simulation model for use in the PK tool of the invention. This method permits the PK tool of the invention to accurately predict one or more *in vivo* pharmacokinetic properties of a compound in a mammalian system of interest from input data derived from a selected *in vitro* or *in vivo* data source. The method includes the steps of (i) generating initial adjustment parameter values for one or more independent parameters of the simulation model by utilizing a curve-fitting algorithm to simultaneously fit to the model one or more input variables corresponding to a pharmacokinetic property of a compound test set derived from (a) a first data source corresponding to the mammalian system of interest, and (b) a second data source corresponding to a system other than the mammalian system of interest; (ii) selecting adjustment parameter values that permit correlation of one or more of the input variables from the first data source to one or more input variables from the second data source; (iii) repeating steps (i) and (ii) one or more times for one or more additional independent parameters of the simulation model until deviation of the correlation is minimized; and (iv) utilizing the selected adjustment parameters as constants for the independent parameters in the simulation model.

The present invention further includes a method for producing a pharmacokinetic-based simulation model for use in the PK tool that facilitates estimation of a selected parameter value in a first segment of mammalian system of interest utilizing input data for the selected parameter that corresponds to a second segment of the mammalian system of interest. The method involves (i) providing a logic function module in the simulation model that includes a set of regional correlation parameter values for at least first and second segments of the mammalian system of interest that facilitates estimation of a selected parameter value in the first segment of the mammalian system of interest utilizing input data for the selected parameter that corresponds to the second segment of the mammalian system of interest; and (ii) providing a control statement in the simulation model which initiates the regional correlation estimation function of the logic function module when a value for the first segment is not supplied as input into the model.

The present invention also provides a method for generating formulation profiles for a compound of interest utilizing the PK tool of the invention.

The PK tool of the invention may be provided as a computer system, as an article of manufacture in the form of a computer-readable medium, or a computer program product and the like. Subsystems and individual components of the PK tool also can be utilized and adapted in a variety of disparate applications for predicting the fate of an administered compound. The PK tool and methods of the invention can be used to screen and design compound libraries, select and design drugs, as well as predict drug efficacy in mammals from *in vitro* and/or *in vivo* data of one or more compounds of interest. The PK tool and methods of the invention also finds use in selecting, designing, and preparing drug compounds, and multi-compound drugs and drug formulations (i.e., drug delivery system) for preparation of medicaments for use in treating mammalian disorders.

DEFINITIONS

Absorption: Transfer of a compound across a physiological barrier as a function of time and initial concentration. Amount or concentration of the compound on the external and/or internal side of the barrier is a function of transfer rate and extent, and may range from zero to unity.

Bioavailability: Fraction of an administered dose of a compound that reaches the sampling site and/or site of action. May range from zero to unity. Can be assessed as a function of time.

Compound: Chemical entity.

Computer Readable Medium: Medium for storing, retrieving and/or manipulating information using a computer. Includes optical, digital, magnetic mediums and the like; examples include portable computer diskette, CD-ROMs, hard drive on computer etc. Includes remote access mediums; examples include internet or intranet systems. Permits temporary or permanent data storage, access and manipulation.

Data: Experimentally collected and/or predicted variables. May include dependent and independent variables.

Dissolution: Process by which a compound becomes dissolved in a solvent.

Input/Output System: Provides a user interface between the user and a computer system.

Permeability: Ability of a physiological barrier to permit passage of a substance. Refers to the concentration-dependent or concentration-independent rate of transport (flux), and collectively reflects the effects of characteristics such as molecular size, charge, partition coefficient and stability of a compound on transport. Permeability is substance and barrier specific.

Physiologic Pharmacokinetic Model: Mathematical model describing movement and disposition of a compound in the body or an anatomical part of the body based on pharmacokinetics and physiology.

Production Rule: Combines known facts to produce ("infer") new facts. Includes production rules of the "IF … THEN" type.

Simulation Engine: Computer-implemented instrument that simulates behavior of a system using an approximate mathematical model of the system. Combines mathematical model with user input variables to simulate or predict how the system behaves. May include system control components such as control statements (e.g., logic components and discrete objects).

Solubility: Property of being soluble; relative capability of being dissolved.

Transport Mechanism: The mechanism by which a compound passes a physiological barrier of tissue or cells. Includes four basic categories of transport: passive paracellular, passive transcellular, carrier-mediated influx, and carrier-mediated efflux.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1 shows schematic of method to generate input data for selected route of administration, mammalian system, and at least one primary barrier to absorption.

Figure 2 shows schematic of method for selecting sampling site relative to administration site and barrier to absorption.

Figure 3 is a high level INPUT/PROCESS/OUTPUT diagram of the PK tool of the invention.

Figure 4 is a high level flow chart and structure chart of the PK tool and method of the invention.

Figure 5 is a graphical diagram illustrating generic compartment-flow simulation model and exemplary symbolic relationships among compartments, flow regulators, converters and input links.

Figure 6 is a key for **Figure 5**.

Figure 7 is a graphical diagram illustrating generic pharmacokinetic first-order two-compartment open plasma model for intravenous injection. D is total drug, V is apparent volume of distribution, and C is drug concentration for either plasma (p) or tissue (t). k12 and k21 represent first-order rate transfer constants for movement of drug from compartment 1 to compartment 2 (k12) and from compartment 2 to compartment 1 (k21). k10 represents first-order rate transfer constant for movement (elimination) of drug from compartment 1 to compartment 0.

Figure 8 is a graphical compartment-flow diagram illustrating the plasma simulation model of Figure 7 and exemplary relationships among compartments, flow regulators, converters and input links.

Figure 9 shows schematic of a method of the invention for development of an initial physiologic-based simulation model for PK tool and method of the invention.

Figure 10 shows schematic of a method of the invention for development of a physiologic-based simulation model having selectively optimized adjustment parameters.

Figure 11 shows graphical compartment-flow diagram illustrating the mass-volume GI tract simulation model of the invention linked to a training/validation plasma model.

Figure 12 illustrates compartment, flow regulator and converter components of the mass-volume GI tract simulation model of the invention.

Figure 13 illustrates structural relationship among compartment and flow regulator components for the mass-volume GI tract simulation model of the invention.

Figure 14 illustrates structural relationship among flow regulator and converter components for the mass-volume GI tract simulation model of the invention.

Figure 15 illustrates converter components for the mass-volume GI tract simulation model of the invention.

Figure 16 compares plasma concentration profiles derived from clinical studies of gancyclovir and simulation using volume GI tract simulation model of the invention.

Figure 17 compares plasma concentration profiles derived from clinical studies of gancyclovir and simulation using mass-volume GI tract simulation model of the invention.

Figure 18 shows graphical compartment-flow diagram illustrating the *in vivo* data analysis-processing IV/PO PK model (intravenous/oral administration) of the invention.

Figure 19 shows schematic of method for development of initial integrated physiologic-based GI tract simulation model of PK tool and method of the invention.

Figure 20 shows graphical compartment-flow diagram illustrating the GI tract fluid transit model component of the PK tool and method of the invention.

Figure 21 shows graphical compartment-flow diagram illustrating the GI tract solubility-dissolution model component of the PK tool and method of the invention.

Figure 22 shows graphical compartment-flow diagram illustrating the GI tract absorption model component of the PK tool and method of the invention.

Figure 23 shows graphical compartment-flow diagram illustrating integration of the GI tract fluid transit model, solubility-dissolution model, and absorption model components for one GI segment of the PK tool and method of the invention.

Figure 24 shows graphical compartment-flow diagram illustrating integrated GI tract simulation model components (without converters or input link connectors) of the PK tool and method of the invention.

Figure 25 shows graphical compartment-flow diagram illustrating integrated GI tract simulation model components (with converters and input link connectors) of the PK tool and method of the invention.

Figure 26 shows schematic of method for development of selectively optimized adjustment parameters and for optimization of the integrated physiologic-based GI tract simulation model of PK tool and method of the invention.

Figure 27 shows schematic of method for selection of model parameters for utilization in a given physiologic-based GI tract simulation model of PK tool and method of the invention.

Figure 28 shows schematic of method for regional (segmental) calculation/estimation of permeability from one or more user input values for permeability of a given GI tract region/segment. Regional permeability (Pe) correlation based on input of Pe value for duodenum is illustrated.

Figure 29 shows graphical converter diagram illustrating volume, surface area, dose, time and pH parameters and calculations for integrated GI tract simulation model components of the PK tool and method of the invention.

Figure 30 shows graphical converter diagram illustrating GI tract transit time parameters and calculations for integrated GI tract simulation model components of the PK tool and method of the invention.

Figure 31 shows graphical converter diagram illustrating GI tract permeability parameters and calculations for integrated GI tract simulation model components of the PK tool and method of the invention.

Figure 32 shows graphical converter diagram illustrating GI tract solubility parameters and calculations for integrated GI tract simulation model components of the PK tool and method of the invention.

Figure 33 shows graphical converter diagram illustrating GI tract control release formulation parameters and calculations for integrated GI tract simulation model components of the PK tool and method of the invention.

Figure 34 shows graphical compartment-converter diagram illustrating GI tract concentration parameters and calculations for integrated GI tract simulation model components of the PK tool and method of the invention.

Figure 35 shows graphical compartment-converter diagram illustrating GI tract dissolution parameters and calculations for integrated GI tract simulation model components of the PK tool and method of the invention.

Figure 36 shows graphical compartment-converter diagram illustrating GI tract output calculations for absorption for integrated GI tract simulation model components of the PK tool and method of the invention.

Figure 37 shows graphical converter diagram illustrating GI tract output calculations for soluble mass absorption rate (flux) for integrated GI tract simulation model components of the PK tool and method of the invention.

Figure 38 shows graphical compartment-flow-converter diagram illustrating GI tract output calculations for cumulative dissolution rate and amount for integrated GI tract simulation model components of the PK tool and method of the invention.

Figure 39 shows graphical compartment-flow-converter diagram illustrating GI tract output calculations for cumulative control release formulation rate and amount for integrated GI tract simulation model components of the PK tool and method of the invention.

Figure 40 illustrates database and rulebase compartment, flow regulator and converter components for the integrated physiologic-based GI tract simulation model of the invention.

Figure 41 illustrates structural relationship among compartment and flow regulator components for the integrated physiologic-based GI tract simulation model of the invention.

Figure 42 illustrates structural relationship among flow regulator and converter components for the integrated physiologic-based GI tract simulation model of the invention.

Figure 43 illustrates structural relationship among converter components for the integrated physiologic-based GI tract simulation model of the invention.

Figure 44 is a high level INPUT/PROCESS/OUTPUT diagram of the PK tool of the invention as presented to a user of the carrying out a method of the invention, with inputs provided by the user and outputs provided by the PK tool.

Figure 45 illustrates a flow chart and structure chart of a subsystem of the PK tool and method of the invention for selection of a physiological GI tract model from a model database and a parameter database.

Figure 46 is a flow chart and structure chart of the system of the PK tool and method of the invention.

Figure 47 is a flow chart and structure chart of a menu of the system of the PK tool and method of the invention.

Figure 48 illustrates correlation of extent of absorption for fraction of the dose absorbed in portal vein (FDp), as predicted using physiologic-based GI tract simulation model and PK tool of the invention, to FDp derived from human clinical data for 12 compounds.

Figure 49 illustrates correlation of rate of absorption for fraction of the dose absorbed in portal vein (FDp), as predicted using integrated physiologic-based GI tract simulation model and PK tool of the invention, to FDp derived from human clinical data for 12 compounds.

Figure 50 compares plasma levels as predicted using integrated physiologic-based GI tract simulation model and PK tool of the invention, to plasma levels derived from human clinical data for a test compound.

Figure 51 compares plasma levels as predicted using integrated physiologic-based GI tract simulation model and PK tool of the invention, to plasma levels derived from human clinical data for a test compound.

Figure 52 compares plasma levels as predicted using integrated physiologic-based GI tract simulation model and PK tool of the invention, to plasma levels derived from human clinical data for a test compound.

Figure 53 shows high level INPUT/PROCESS/OUTPUT diagram of the PK tool of the invention for SAR/QSAR and CAD/CAE compound design and synthesis.

Figure 54 shows high level flow and structure chart for screening method of the invention utilizing the PK tool and method of the invention.

DESCRIPTION OF SPECIFIC EMBODIMENTS

A pharmacokinetic tool (PK tool) and method is provided for predicting absorption of a compound relative to a physiological barrier of a mammalian system of interest, including extravascularly administered compounds. This includes, but is not limited to, prediction of rate, extent and/or concentration of a compound. The mammal is a human or a non-human animal. The method utilizes the PK tool, and optionally separately operable subsystems or components thereof. The PK tool and method of the invention also facilitates prediction of the fate of a compound in a mammal based on absorption and one or more additional bioavailability parameters including distribution, metabolism, elimination, and optionally toxicity.

The PK tool includes as computer-readable components, an input/output system, a physiologic-based simulation model of a mammalian system of interest, and a simulation engine. The input/output system may be any suitable interface between user and computer system, for input and output of data and other information, and for operable interaction with a simulation engine and a simulation model.

Input data into the PK tool and method of the invention is dose, permeability and solubility data for a test compound of interest, and optionally one or more of dissolution rate, transport mechanism, transit time, pH, delivery system rate such as controlled release rate or formulation release rate (delivery system referred to herein as "formulation"), dosing schedule, and simulation run time. The input data may be derived from *in vitro* or *in vivo* sources. *In vitro* data includes tissue and cell and natural and artificial preparations thereof, physicochemical, molecular structure and molecular structure-activity relationship (SAR) and quantitative-SAR (QSAR) data. *In vivo* data includes mammal data. The input data corresponds to one or more given physiological segments/regions of the mammalian system of interest.

The simulation output includes an absorption profile characterized by one or more of rate of absorption, extent of absorption, and concentration of the compound relative to a selected sampling site of interest located across a physiological barrier of the mammalian system of interest, i.e., rate and/or extent of transfer of a test sample from an external site (e.g., apical) across a physiological barrier (e.g., epithelium) to an internal site (e.g., basolateral) of that barrier. This can include prediction of rate, extent and/or concentration of a compound at the site of action when the selected sampling site is the site of action. Transfer rate and/or extent are generated utilizing initial dose data for the test compound and *in vitro* and/or *in vivo* derived data including permeability and solubility data, and optionally dissolution rate and transport mechanism data (i.e., passive paracellular, passive transcellular, carrier-mediated influx, carrier-mediated efflux) for the test compound. Solubility and dissolution rate are interrelated and effect the ability of the compound to be solubilized at a rate sufficient for absorption to occur across a particular membrane. Permeability refers to the concentration-dependent or concentration-independent rate of transport (flux), and collectively reflects the effect of molecular size, charge, partition coefficient and stability of a compound on absorption for a particular physiological barrier, where the physiological barrier(s) depends on the selected route of administration. Molecular size, charge and partition coefficient determines in large part whether a compound is transported via a paracellular or transcellular mechanism. Stability is a general feature that relates to whether the compound remains intact long enough to be absorbed. Together, dose, solubility and permeability data, and optionally dissolution rate and transport mechanism data, are primary bioavailability factors utilized by the PK tool and method of the invention to generate an absorption profile for a test compound of interest.

An absorption profile generated by the PK tool and method of the invention can be uni- or multi-dimensional output that reflects one or more simulated parameters of the mammalian system of interest relative to the sampling site. The sampling site, for example, portal vein, plasma, tissue, organ and the like, is chosen depending on the intended end use of the PK tool and method of the invention. Output of the method and PK tool can be utilized to profile or rank the compound by a selected absorption parameter, and optionally, absorption and one or more additional bioavailability parameters and toxicity.

The simulation engine comprises a differential equation solver and, optionally, a system control statement module. This includes various computer-readable algorithms for numerical iteration of mathematical equations over interval dt and for processing rules, scenarios and the like that direct a simulation.

The simulation model corresponds to a physiologic-based multi-compartment model of a mammalian system of interest, where the mammalian system represents a physiological barrier to absorption that is based on a selected route of administration, i.e., the location at which the compound is introduced to a mammal. More particularly, the physiologic-based simulation model of the PK tool and method of the invention is a mathematical model comprising as operably linked components: (i) differential equations for calculating one or more of fluid transit, fluid absorption, mass transit, mass dissolution, mass solubility, and mass absorption of a test compound for one or more physiological segments of the mammal system of interest; and (ii) initial parameter values for the differential equations corresponding to physiological parameters and selectively optimized adjustment parameters, and optionally one or more regional correlation parameters, for one or more physiological segments of the mammal system of interest; and optionally (iii) control statement rules for one or more of absorption, permeability, solubility, dissolution, concentration, and mathematical error correction, for one or more physiological segments of the mammal system of interest. The simulation model also may include one or more smoothing functions that facilitate calculation of transitional parameter values occurring between one or more of the physiological segments.

The differential equations of a selected simulation model of a mammalian system of interest describe the rate processes of absorption, and optionally other events, of that model, which in turn describe compound concentrations in the system as a function of time. (See, e.g., Shargel et al., *Applied Biopharmaceutics and Pharmacokinetics,* Appelton & Lange, East Norwalk, Conneticut, 1993). Thus, the differential equations are selected for a particular model.

The initial physiological parameter values of a given simulation model can be generated de novo or obtained from existing sources including the literature. The selectively optimized adjustment parameter values of a given simulation model of the invention represent regression or stochastic analysis derived values that are used as constants for one or more independent parameters of the model. In particular, the selectively optimized adjustment parameter values are obtainable by using a stepwise fitting and selection process that employs regression- or stochastic-based curve-fitting algorithms to simultaneously estimate the change required in a value assigned to an initial absorption parameter of the model in order to change an output variable. The input variables utilized for fitting include a combination of *in vitro* data (e.g., permeability, solubility) and *in vivo* pharmacokinetic data (e.g., fraction of dose absorbed, plasma levels) for a compound test set having compounds exhibiting a diverse range of *in vivo* absorption properties. Thus, the input variables used for regression- or stochastic-based fitting are derived from (a) a first data source corresponding to the mammalian system of interest (e.g., *in vivo* pharmacokinetic data from human for the compound test set), and (b) a second data source corresponding to a system other than the mammalian system of interest (e.g., *in vitro* solubility data and *in vitro* permeability data from rabbit tissue for the compound test set). A fitted adjustment parameter value for a given independent parameter is then selected that, when supplied as a constant in the model, permits correlation of one or more of the input variables from the first data source to one or more input variables from the second data source. The process is repeated one or more times for one or more additional independent parameters of the simulation model until deviation of the correlation is minimized. These "selectively optimized" adjustment parameters are then provided to a given simulation model as constants or ranges of constants or functions that modify the underlying equations of the model. The selectively optimized adjustment parameters facilitate accurate correlation of *in vitro* data derived from a particular type of assay corresponding to the second data source (e.g., Caco-2 cells, segment-specific rabbit intestinal tissue sections etc.) to *in vivo* absorption for a mammalian system of interest corresponding to the first data source (e.g., segment-specific portions of the human GI tract) for diverse test sample data sets. Selectively optimized adjustment parameters also can be utilized to facilitate accurate correlation of *in vivo* data derived from a first species of mammal (e.g., rabbit) to a second species of mammal (e.g., human).

For a simulation model representing two or more anatomical segments of a given mammalian system, the model will preferably include regional correlation parameters. The regional correlation parameters permit estimation of a selected parameter value for a first segment of the mammalian system from correlation using a value of the selected parameter for a second segment of the mammalian system. The regional correlation parameters represent a collection of empirically derived values or selectively optimized adjustment parameter values for various segments of the mammalian system of interest, for example, permeability values. The regional (i.e., segmental) correlation is performed by logic function of the model, which when activated utilizes a function/transformation algorithm to estimate the parameter value for the second segment from (1) the corresponding regional correlation parameters, and (2) a user provided input value for the same parameter, but for a different segment. The regional correlation logic function of the model is activated when a user does not supply an input value for a particular parameter. For example, when a user of the PK tool supplies a single permeability value as input into a GI tract simulation model of the invention, such as a permeability value derived from Caco-2 cells that corresponds to colon, then regional permeability correlation is performed by the PK tool to estimate permeability in the other GI tract segments, such as duodenum, jejunum, and ileum.

The control statement rules include various logic elements utilized for providing guidance as to how a given simulation is to proceed. For instance, a control statement rule would include "IF ... THEN" production rules. An example of a production rule would be "IF solubility of compound is zero THEN absorption is zero." The production rules are based on rules of thumb (heuristics) and the like, and may be generated by correlation of parameters and simulation runs. Rules can be added, modified or removed to change how a simulation model responds to incoming data.

The input/output system, simulation engine and simulation model of the PK tool are capable of working together to carry out the steps of (1) receiving as input data, the initial dose of a test compound at the site of administration and permeability and solubility, and optionally dissolution rate and transfer mechanism data; and (2) applying the simulation engine and the simulation model to generate as output data a simulated *in vivo* absorption profile for the test compound that reflects rate, extent and/or concentration of the test sample at a given sampling site for a selected route of administration in a mammalian system of interest. This includes uni- and multi-dimensional output profiles that collectively reflect parameters of absorption, which can be directly or indirectly utilized for characterizing *in vivo* absorption, as well as one or more additional bioavailability parameters including distribution, metabolism, elimination, and optionally toxicity.

The selected routes of administration include enteral (e.g., buccal or sublingual, oral (PO), rectal (PR)), parenteral (e.g., intravascular, intravenous bolus, intravenous infusion, intramuscular, subcutaneous injection), inhalation and transdermal (percutaneous). The preferred route of administration according to the method of the invention is oral administration. The selected route of administration determines the type and/or source of assay or structure-property parameters employed for obtaining a set of input data utilized for generating a simulated *in vivo* absorption profile. That is, artificial, cell or tissue preparations and the like derived from or representative of a physiological barrier to absorption for a selected route of administration are chosen to generate the relevant input data for use as input into the PK tool. For instance, input data for simulating fate of a test sample following oral administration can be based on cell culture and/or tissue assays that employ biological preparations derived from or representative of the gastrointestinal tract of a mammal of interest, e.g., gastrointestinal epithelial cell preparations for permeability and transfer mechanism data, and physiological/anatomical fluid and admixing conditions corresponding to the relevant portions of the gastrointestinal tract for solubility and dissolution rate assays. Assays for collecting input data for specialized physiological barriers such as the blood brain barrier may initially assume intravascular delivery and thus instantaneous absorption as a first step. In this situation an assay is selected to generate input data relative to the blood brain barrier, which include for instance cell culture and/or tissue assays that employ biological preparations derived from or representative of the interface between systemic blood and the endothelial cells of the microvessels of the brain for a mammal of interest, e.g., blood-brain-barrier microvessel endothelial cell preparations for permeability and transfer mechanism data, and physiological/anatomical fluid and admixing conditions corresponding to the relevant portions of the blood membrane barrier for solubility and dissolution rate assays. A series of assays may be employed to collect input data for two or more barriers to absorption. As an example, oral, hepatic, systemic and blood brain barrier assays may be utilized to obtain input data for screening compound libraries for orally delivered compounds that target brain tissue.

The sampling site relates to the point at which absorption parameters are evaluated for a test sample of interest. This is the site at which rate, extent and/or concentration of a test sample is determined relative to a selected site of administration, and is separated from the site of administration by at least one physiological barrier to absorption. For generating simulated absorption profiles, the sampling site preferably is separated from the site of administration by an individual primary barrier to absorption, which can be utilized to evaluate additional absorption events by secondary barriers to absorption so as to sequentially and collectively reflect the summation of absorption events at other sampling sites of interest. As an example, the sampling site selected for oral delivery may be the portal vein where the primary barrier to absorption is the gastrointestinal lumenal membrane, or systemic blood where a secondary barrier to systemic absorption is the liver after the test sample passes from the portal vein through the liver to systemic circulation. Thus the type of physiological barrier(s) residing between a site of administration and a sampling site reflects the type of assay(s) employed for generating the desired input data for use as input data into the PK tool of the invention.

As the selected route of administration determines the barrier(s) to absorption and the physiological parameters that affect absorption events following administration, it also determines the physiologic-based pharmacokinetic simulation model employed in the PK tool for generation of the simulated *in vivo* absorption profile. By way of example, if the proposed route of administration is oral, then a primary barrier to absorption is the lumenal membrane of the gastrointestinal tract, and a secondary event affecting systemic bioavailability is first pass metabolism by the liver. Thus, a given simulation model and its associated parameters for simulating the fate of a compound selected for oral delivery is chosen to represent this scenario. The model would include therefore relevant components of the gastrointestinal tract for administration and absorption (i.e., stomach, duodenum, jejunum, ileum, and colon) and a primary sampling site (i.e., portal vein) from which to evaluate a primary absorption event. In this instance a secondary barrier to absorption for oral delivery is the liver and a secondary sampling site is systemic blood/plasma. This basic approach to choosing a physiologic-based pharmacokinetic model also applies to models employed to simulate absorption by target organs and the like, where a physiological barrier to absorption is the tissue and/or membrane separating systemic blood from the target organ, such as the blood brain barrier. In this situation if oral delivery is selected as the preferred route of administration for a compound targeting brain tissue, then a gastrointestinal tract model and blood brain barrier model may be implemented separately and/or combined through a complementary plasma component of the models for screening purposes.

The physiological models are selected from a repository of delivery route-specific models stored in a memory, a database, or created de novo. Physiological models of the invention include those corresponding to common routes of administration or barriers to absorption, such as oral (GI tract), ocular (eye), transdermal (skin), rectal, intravenous, rectal, subcutaneous, respiratory (nasal, lung), blood brain barrier and the like. For constructing a model de novo, the basic approach is to identify and isolate a primary barrier to a specific absorption event from secondary events so that each barrier to absorption can be tested and validated in isolation. This involves selecting a site of administration that is separated from a sampling site by a primary physiological barrier to absorption and then building a developmental physiological model that incorporates rate process relations and limitations to describe the isolated absorption event. If desired, the secondary events can be added sequentially so that each additional layer of complexity to the model can be tested and validated in isolation from other components of the initial model.

The invention also relates to a method and PK tool for designing compounds based on absorption. This aspect of the invention utilizes output of the method and PK tool as the input to a structure-activity relationship (SAR) or quantitative SAR (QSAR) design/selection process, e.g., a SAR and/or QSAR computer-assisted design/engineering/selection (CAD/CAE (collectively "CAD")) process. Output of the CAD process is then optionally used as input for the method and PK tool of the invention. SAR and QSAR information may then be incorporated into a database for subsequent iterative design and selection in the CAD process. For instance, compounds designed using a CAD process may be tested *in vitro* and/or *in vivo* for absorption parameters such as permeability, solubility, dissolution, and transport mechanism, and optionally one or more additional bioavailability parameters, and the data employed as input into the PK tool and method of the invention (i.e., iterative design). Alternatively, the parameters can be predicted from SAR or QSAR information and utilized as input for the method and PK tool of the invention. In this aspect of the invention, the user also is allowed to vary input parameters for "What if" analysis.

In the forward mode of operation, the user can predict absorption, individual parameters of absorption, as well as one or more other bioavailability parameters of a compound from relatively few input variables including dose, permeability and solubility. Additionally, the user can evaluate alternatives by changing any of the parameters and constants of the system, and observe the ripple effect of the change in one or more parameters on all other parameters. For instance, the user can evaluate alternative absorption profiles using "What if" analysis with any parameter of the system.

In the backward mode of operation, the user specifies one or more objective absorption parameters of a formulation of interest and the PK tool and method of the invention generates alternatives to satisfy the objective. In this aspect of the invention, well-defined properties of the compound (and the formulation base minus the compound) are utilized by the PK tool and method to evaluate alternative dosing and formulation profiles for a given compound. The user also is allowed to vary input dosing and formulation parameters for "What if" analysis. Simulated absorption profiles can then be utilized for preparing suitable formulations and/or dosing regimes. Solubility, permeability, doses and the like also may be estimated in the backward mode of operation.

The PK tool and method of the invention is exemplified by physiologic-based simulation model for predicting oral absorption of a compound in one or more segments of the GI tract of a mammal. The segments include the stomach, duodenum, jejunum, ileum, and colon. The simulation model includes differential equations for calculating one or more of fluid transit, fluid absorption, mass transit, mass dissolution, mass solubility, and mass absorption for one or more segments of the GI tract of a mammal of interest. It also includes initial parameter values for the differential equations that correspond to physiological parameters and selectively optimized adjustment parameters for one or more segments of the GI tract of the mammal of interest. The initial parameter values of simulation model also include one or more regional correlation parameter values, which are optional, but preferred for inclusion. The simulation model of the GI tract also includes control statement rules for one or more of transit, absorption, permeability, solubility, dissolution, concentration, and mathematical error correction for one or more segments of the GI tract of the mammal of interest.

The physiologic-based simulation model of the GI tract corresponds to a compartment-flow simulation model of the GI tract of a mammal characterized by one or more of fluid volume, fluid absorption, insoluble mass, soluble mass, and soluble mass absorption compartments. The compartments of the compartment-flow simulation model are operably linked by one or more flow regulators characterized by fluid absorption rate, fluid volume transit rate, insoluble mass transit rate, insoluble mass dissolution rate, soluble mass transit rate, and soluble mass absorption rate. The flow regulators of the compartment-flow simulation model are modified by one or more converters characterized by fluid volume, fluid volume absorption rate constant, fluid volume transit rate constant, insoluble mass, insoluble mass transit rate constant, dissolution rate constant, soluble mass, soluble mass transit rate constant, surface area, dissolved mass concentration and permeability.

The PK tool and method of this invention accelerate selection and design of compounds for treatment of mammalian disorders, allowing same day response time. The invention optimizes the drug development process in terms of bioavailability parameters, and uses simple *in vitro* parameters for predicting the *in vivo* fate of an administered compound. The PK tool and method of the invention also permits utilization of *in vivo* data from one type of mammal (e.g. rabbit) to predict absorption in a different type of mammal (e.g. human). The invention also is particularly well suited for iterative selection and design of compounds based on structure-bioavailability relationships using a SAR/QSAR approach. This reduces total drug development time, and optimizes the drug design and selection process for animal studies and human clinical trials. Moreover, the PK tool and method of the invention allows separate or concurrent consideration of bioavailability parameters and/or biological drug-receptor activity early in the drug development process. The invention also permits a broad range of *in vitro* to interspecies correlation, thereby facilitating optimal selection of an animal model for drug development.

PK Tool and System:

The PK tool of the invention is utilized to generate a simulated *in vivo* absorption profile from dose, solubility and permeability data, and optionally *in vitro* dissolution rate and transport mechanism data for a test compound. The PK tool includes as computer-readable components, an input/output system suitable for data input and data output, a simulation engine having a differential equation solver, and a physiologic-based simulation model comprising a pharmacokinetic model of the mammalian system to be simulated. *In vitro* or *in vivo* data for the test compound is provided through the input/output system, and then the simulation engine and simulation model are applied to facilitate a simulation run so as to generate a user selected *in vivo* absorption profile for the test sample. Together, the simulation engine and simulation model are employed to simulate the fate of a test sample in the system under investigation.

The PK tool is based on a compartment-flow simulation model system. The compartment-flow model employs compartments, flow regulators, and converters that collectively regulate flow among the compartments. The model components are represented by a series of differential equations which when run through the simulation engine are solved at each time increment dt based on the initial underlying values of the equations, the input values supplied by the user, and calculations performed by various subsystems of the model when activated in a particular scenario.

The PK tool optionally comprises a repository of different pharmacokinetic models and initial parameter values for a given model. The repository preferably resides in a database of the PK tool, and/or is accessible through an acquisition module. The PK tool also may include one or more curve-fitting algorithms for generation of absorption parameters and constants for correlation of *in vitro* data to *in vivo* data, or *in vivo* data from one species of a mammal to *in vivo* data of a second species of mammal based on a selected route of administration. The curve-fitting algorithms include regression-based and stochastic-based algorithms.

1. Input/Output System

With regard to the components of the PK tool, the input/output system provides a user interface between the user and the PK tool of the invention. The input/output system may be any suitable interface between user and computer system, for input and output of data and other information, and for operable interaction with a simulation engine and a simulation model. For instance, the input/output system may provide direct input form measuring equipment. The input/output system preferably provides an interface for a standalone computer or integrated multi-component computer system having a data processor, a memory, and a display. Input into the method and PK tool of the invention is *in vitro* or *in vivo* data derived from an assay corresponding to a selected route of administration and mammalian system of interest. For example, the user enters the initial parameter values for a test compound, e.g., dose, permeability, and solubility derived from the assay, and then optionally indicates the transport mechanism, e.g., passive transcellular, passive paracellular, carrier-mediated influx, or carrier-mediated efflux. When transport mechanism is not indicated, the PK tool can be designed to employ a default transport mechanism, such as passive transcellular. When set to the paracellular mechanism, the absorption of the compound is adjusted to compensate for the lower surface area available for absorption via the paracellular pathway. The model also may incorporate an operation by which the mechanism of absorption can be predicted using the permeability, solubility, molecular structure or other information. This allows the model to automatically compensate for paracellular and/or other absorption mechanisms without requiring prior input and knowledge from the user. Depending on the objective, the user also may specify the pH, delivery system rate such as controlled release rate or formulation release rate (delivery system referred to herein as "formulation"), dosing schedule, and simulation run time, as well as physiologic system specific parameters such as GI transit time when a GI tract model is employed. If values for these parameters are not entered, the PK tool provides default values.

Data may be entered numerically, as a mathematical expression or as a graph that represents a physiological or pharmacokinetic parameter, or alpha such as transcellular, paracellular, passive, active, etc. An advantage of entering data as a graph is that it removes any requirement to define the mathematical relationship between a dependent and an independent variable. The interface output displays and/or compares parameters related to absorption, such as graphs or tables corresponding to rate of absorption, extent of absorption, and concentration profiles, and the like.

The absorption parameters include concentration, rate and/or extent of absorption of a test sample. As can be appreciated, absorption parameters can be represented in multiple different ways that relate time, mass, volume, concentration variables, fraction of the dose absorbed and the like. Examples include rate "dD/dt" and "dc/dt" (e.g., mass/time-mg/hr; concentration/time-μg/ml/hr), concentration "C" (e.g., mass/volume-μg/ml), area under the curve "AUC" (e.g., concentration • time, μg • hr/ml), and extent/fraction of the dose absorbed "F" (e.g., no units, 0 to 1). Other examples include the maximum concentration (C_{max}), which is the maximum concentration reached during the residence of a compound at a selected sampling site; time to maximum concentration (T_{max}), which is the time after administration when the maximum concentration is reached; and half-life ($t_{1/2}$), *e.g.*, the time where the concentration reaches ½ its maximum at a selected sampling site. Other examples of output include individual simulated parameters such as permeability, solubility, dissolution, and the like for individual segments, as well as cumulative values for these and/or other parameters.

2. Simulation Engine

The simulation engine comprises a differential equation solver that uses a numerical scheme to evaluate the differential equations of a given physiologic-based simulation model of the invention. The simulation engine also may include a system control statement module when control statement rules such as IF...THEN type production rules are employed. The differential equation solver uses standard numerical methods to solve the system of equations that comprise a given simulation model. These include algorithms such as Euler's and Runge-Kutta methods. Such simulation algorithms and simulation approaches are well known (See, e.g., Acton, F.S., *Numerical Methods that Work*, New York, Harper & Row (1970); Burden et al., *Numerical Analysis*, Boston, MA, Prindle, Weber & Schmidt (1981); Gerald et al., Applied Numerical Analysis, Reading, MA, Addison-Wesley Publishing Co., (1984); McCormick et al., Numerical Methods in Fortran, Englewood Cliffs, NJ, Prentice Hall, (1964); and Benku, T., The Runge-Kutta Methods, BYTE Magazine, April 1986, pp. 191-210).

Many different numerical schemes exist for the evaluation of the differential equations. There are literally 100's of schemes that currently exist, including those incorporated into public commercially available computer applications, private industrial computer applications, private individually owned and written computer applications, manual hand-calculated procedures, and published procedures. With the use of computers as tools to evaluate the differential equations, new schemes are developed annually. The majority of the numerical schemes are incorporated into computer applications to allow quick evaluation of the differential equations.

Computer application or programs described as simulation engines or differential equation solver programs can be either interpretive or compiled. A compiled program is one that has been converted and written in computer language (such as C++, or the like) and are comprehendible only to computers. The components of an interpretive program are written in characters and a language that can be read and understood by people. Both types of programs require a numerical scheme to evaluate the differential equations of the model. Speed and run time are the main advantages of using a compiled rather than a interpretive program.

A preferred simulation engine permits concurrent model building and simulation. An example is the STELLA® program (High Performance Systems, Inc.). STELLA® is an interpretive program that can use three different numerical schemes to evaluate the differential equations: Euler's method, Runge-Kutta 2, or Runge-Kutta 4. The program KINETICA™ (InnaPhase, Inc.) is another differential equation solving program that can evaluate the equations of the model. By translating the model from a STELLA® readable format to a KINETICA™ readable format, physiological simulations can be constructed using KINETICA™, which has various fitting algorithms. This procedure can be utilized when the adjustment parameters are being optimized in a stepwise fashion.

3. Simulation Model

The simulation model is a mathematical model of a multi-compartment physiological model of a mammalian system (e.g., GI tract) that corresponds to the selected route of administration (e.g., oral). A given physiological model is represented by series of differential equations that describe rate process interactions among anatomical segments for the physiological system under investigation. The individual segments or compartments are represented mathematically as a one, two and/or three compartment kinetic system. The segments are linked in a stepwise fashion so as to form an integrated physiological model describing absorption of a compound relative to the anatomical segments and at least one sampling site for assessing an absorption event in isolation. For a model simulating oral delivery, anatomical segments of the GI tract are provided, which can include the stomach, duodenum, jejunum, ileum and colon. A sampling site for the GI tract may be the portal vein and/or plasma. The rectum and colon would be applicable for modeling a rectal route of delivery. Segments and sampling site for buccal or sublingual delivery routes can include the mouth, cheek/tongue tissue and plasma. For ocular routes, this can include aqueous humor, conjunctival sac, tear duct, nasal cavity and plasma. For the lung routes, this can include respiratory bronchioles zone and plasma. For delivery via the nose, this can include nasal cavity and plasma. For the topical and transdermal routes, this can include epidermal, dermal, subcutaneous tissue, muscle and plasma. Other systems adhere to these basic designs.

Of course compartments representing a particular anatomical segment can be added or removed depending on the model's intended end use, such as when an isolated segment is examined, or when it is desired to account for parameters affecting bioavailability at additional sampling sites. For example, compartments can be added to account for both pre- or post-absorptive protein binding or complex formation to account for reversible association of a compound to the proteins (albumin and a1-acid glycoprotein) of blood, or more usually plasma. Other compartments that may be added would include those that account for phase I and/or phase II hepatic metabolism. Formulation compartments that account for variable compound formulations also can be added, such as time-release, extended release or otherwise controlled release formulations. Another example is inclusion of kidney compartments to account for renal clearance.

The compartments can be modified by factors that influence absorption such as mass, volume, surface area, concentration, permeability, solubility, fluid secretion/absorption, fluid transit, mass transit and the like, depending on the physiological system under investigation. As a rule of thumb, compartment modifiers relate to input variables. For instance, where transport mechanism and dissolution rate are variables considered for generating an absorption profile, then the physiological model will include compartments and parameters that account for these variables.

When represented as a compartment-flow simulation model, the anatomical segments of a physiological model typically include one or more central and peripheral compartments that reversibly communicate through a flow regulator. A central compartment represents the interior or mucosal side of an anatomical segment. A peripheral compartment represents the blood side of the segment. The central and peripheral compartments are connected by a flow regulator representing a physiological barrier through which material from the central compartment "flows" or is transferred to the peripheral compartment at some empirically defined or calculated transfer rate "k12" applied by a converter, which allows calculation of parameters using compartment values. Transfers ("flows") between compartments can be zero order, first order, second order and/or mixed order processes. As an example, a first order transfer from central compartment 1 to peripheral compartment 2 can be defined by a finite difference equation connecting inputs (e.g., rate constant k12 and amount of compound in central compartment = amount + dt*(-elimination – k12+ k21)) to the flow controller between the compartments (e.g., k12) and setting it as the product of the two variables. Thus the underlying equations of the model are utilized to calculate the amount of a compound in each compartment, and standard differential equations interrelate the system of compartments and their equations. This permits the model to simulate movement of a compound through each compartment according to the calculated rates at each time increment (dt). Since all movement between compartments is in units of mass, the blood side and transferred test compound concentration is calculated from the amount of compound in the blood side (peripheral compartment) and volume of the mucosal side (central compartment). A model cycle is entered through the input/output user interface as incremental pulses (to simulate ramp, plug flow/lag times) or as a fixed time range to initiate and effectuate cycling of a test compound of interest.

The basic structure of a physiological model and mathematical representation of its interrelated anatomical segments can be constructed using any number of techniques. The preferred techniques employ graphical-oriented compartment-flow model development computer programs such as STELLA®, Kinetica™ and the like. Many such programs are available, and most employ graphical user interfaces for model building and manipulation. In essence, symbols used by the programs for elements of the model are arranged by the user to assemble a diagram of the system or process to be modeled. Each factor in the model may be programmed as a numerical constant, a linear or non-linear relationship between two parameters or as a logic statement. The model development program then generates the differential equations corresponding to the user constructed model. For example, STELLA® employs five basic graphic tools that are linked to create the basic structure of a model: (1) stocks; (2) flows; (3) converters; (4) input links; and (5) infinite stocks (See, e.g., Peterson *et al.*, STELLA® II, Technical Documentation, High Performance Systems, Inc., (1993)). Stock are boxes that represent a reservoir or compartment. Flows or flow regulators control variables capable of altering the state of compartment variables, and can be both uni- and bi-directional in terms of flow regulation. Thus, the flow/flow regulators regulate movement into and out of compartments. Converters modify flow regulators or other converters. Converters function to hold or calculate parameter variable values that can be used as constants or variables which describe equations, inputs and/or outputs. Converters allow calculation of parameters using compartment values. Input links serve as the internal communication or connective "wiring" for the model. The input links direct action between compartments, flow regulators, and converters. In calculus parlance, flows represent time derivatives; stocks are the integrals (or accumulations) of flows over time; and converters contain the micro-logic of flows. The stocks are represented as finite difference equations having the following form: Stock(t) = Stock(t-dt) + (Flow)*dt. Rewriting this equation with timescripts and substituting t for dt: $\text{Stock}_t = \text{Stock}_{t-\Delta t} + \Delta t^*(\text{Flow})$. Re-arranging terms: $(\text{Stock}_t - \text{Stock}_{t-\Delta t})/\Delta t = \text{Flow}$, where "Flow" is the change in the variable "Stock" over the time interval "t." In the limit as Δt goes to zero, the difference equation becomes the differential equation: d(Stock)/dt = Flow. Expressing this in integral notation: $\text{Stock} = \int \text{Flow dt}$. For higher-order equations, the higher-order differentials are expressed as a series of first-order equations. Thus, computer programs such as STELLA® can be utilized to generate physiologic-based multi-compartment models as compartment-flow models using graphical tools and supplying the relevant differential equations of pharmacokinetics for the given physiologic system under investigation. An example of iconic tools and description, as well as graphically depicted compartment-flow models generated using STELLA® and their relation to a conventional pharmacokinetic IV model is illustrated in **Figures 5-8.**

The model components may include variable descriptors. Variable descriptors for STELLA®, for example, include a broad assortment of mathematical, statistical, and built in logic functions such as boolean and time functions, as well as user-defined constants or graphical relationships. This includes control statements, e.g., AND, OR, IF … THEN ... ELSE, delay and pulsing, that allow for development of a set of production rules that the program uses to control the model. Variable descriptors are inserted into the "converters" and connected using "input links." This makes it is possible to develop complex rule sets to control flow through the model. The amount of time required to complete one model cycle is accomplished by inputting a total run time and a time increment (dt). The STELLA® program then calculates the value of every parameter in the model at each successive time increment using Runge-Kutta or Euler's simulation techniques. The preferred simulation technique is Runge-Kutta. Once a model is built, it can be modified and further refined, or adapted or reconstructed by other methods, including manually, by compiling, or translated to other computer languages and the like depending on its intended end use.

A preferred method of the invention for constructing a physiological model for *in vivo* prediction from *in vitro* input data is depicted in **Figure 9**. This method employs a two-pronged approach that utilizes a training set of standards and test compounds having a wide range of dosing requirements and a wide range of permeability, solubility, transport mechanisms and dissolution rates to refine the rate process relations and generate the initial values for the underlying equations of the model. The first prong employs the training/validation set of compounds to generate *in vivo* pharmacokinetic data (e.g., human plasma profiles). The second prong utilizes the training/validation set of compounds to generate *in vitro* permeability, solubility, transport mechanism and dissolution rate data that is employed to perform a simulation with the developmental physiological model. The *in vivo* pharmacokinetic data is then compared to the simulated *in vivo* data to determine how well a developmental model can predict the actual *in vivo* values from *in vitro* data. The developmental model is adjusted until it is capable of predicting *in vivo* absorption for the training set from *in vitro* data input. Then the model can then be validated using the same basic approach and to assess model performance.

In particular, three primary sets of data are generated from the training set for the comparison. The first set of data is empirically derived *in vivo* plasma data from animals or humans. The second set of data is obtained from conversion of the *in vivo* plasma data to a form corresponding to the primary sampling site of the developmental physiological model. The third set of data is empirically derived *in vitro* data including permeability, solubility, dissolution rate and transport mechanism data. The raw data points are preferably collected and statistically analyzed to provide the best fit data. The best fit data may be obtained by any number of curve-fitting approaches, including standard regression techniques.

The *in vivo* plasma data is utilized to judge how well a developmental simulation model is able to predict absorption of the training set of compounds relative to the empirically derived *in vivo* plasma values. Plasma data also is utilized to calculate absorption at the relevant primary sampling site of the developmental physiological model. For instance, in order to use *in vivo* plasma data in a developmental physiological model, the plasma data must first be converted to data corresponding to the primary sampling site of the model. If plasma is the primary sampling site then no conversion is needed. However, if plasma is not the primary sampling site, then a pharmacokinetic training/validation model relating the primary sampling site and the *in vivo* plasma data is utilized. For example, when the developmental model is of the gastrointestinal tract, the portal vein can be selected as a primary sampling site and plasma selected as a secondary sampling site. Thus in this instance the *in vivo* plasma data is converted to portal vein data so that the parameters affecting secondary bioavailability events are separated from the primary absorption event resulting from passage of the test sample across the gastrointestinal lumen. This is accomplished by adding a plasma-portal vein conversion/validation model that relates *in vivo* plasma data to portal vein data. This plasma-portal vein conversion/validation model can be separate or integrated with the developmental model. In most cases, the plasma-portal vein model is based on a standard central-peripheral pharmacokinetic compartment approach for data conversion. The third set of data, the *in vitro* derived data, is utilized to run the developmental model, and the simulated absorption profile from this data set is compared to the *in vivo* derived plasma and simulated sampling site data. The developmental physiological model is modified until the simulated absorption profiles are in agreement with the *in vivo* derived plasma and simulated sampling site data.

As the number of parameters for evaluation increase it becomes more important to isolate and test each component of the model building process by validation using a standard validation set of compounds. The validation set of compounds should contain a diverse set of compounds that represent a broad range of absorption profiles for which both *in vitro* permeability, solubility, dissolution rate, and transport mechanism data, and *in vivo* plasma data is available. Statistical criteria such as sum of squares of the deviations between experimental data and calculated values obtained from the developmental physiological model are used to determine how well the model fits the data. If the developmental physiological model does not predict a good fit for the data, then the model is adjusted by isolating or including additional rate processes by an iterative approach.

Parameter values utilized in the underlying equations of a given physiological model may be provided in a database for ready access and manipulation by the PK tool of the invention, or provided with a model. The parameter values may include values for physiological parameters, such as rate constants and various other values employed in the PK tool. The rate constants correspond to time-dependent (or time-independent) numerical constants describing rate processes (e.g., k12 and k21). The physiological parameters include rate constants, permeability, solubility, transport mechanism and dissolution rate variables, and the like, as well as pH, volume, surface area, transit times, transit rates, and the like, that are based on the physiology of a given anatomical segment represented in a selected physiological model.

To account for differences between *in vitro* and *in vivo* conditions, as well as differences between *in vivo* conditions of different type of mammals, adjustment parameters that modify one or more of the underlying equations of given simulation model can be utilized to significantly improve predictability. The adjustment parameters include constants or ranges of constants that are utilized to correlate *in vitro* input parameter values derived from a particular *in vitro* assay system (e.g., rabbit intestinal tissue, Caco-2 cells) to a corresponding *in vivo* parameter value employed in the underlying equations of a selected physiological model (e.g., human GI tract). The adjustment parameters are used to build the correlation between the *in vitro* and *in vivo* situations, and *in vivo* (species 1) to *in vivo* (species 2). These parameters make adjustments to the equations governing the flow of drug and/or calculation of parameters. Generally, the parameters are geometric scaling parameters, as exemplified by the general equations described below for a GI tract simulation model of the invention. This aspect of the invention permits modification of existing physiologic-based pharmacokinetic models as well as development of new ones so as to enable their application for diverse compound data sets.

The adjustment parameters of the PK tool and method of the invention are obtainable from iterative rounds of simulation and simultaneous "adjustment" of one or more empirically derived absorption parameters (e.g., physiological parameters for different anatomical segments) until the *in vitro* data from a given type of assay (e.g., Caco-2 cell data) can be used in the model to accurately predict *in vivo* absorption in the system of interest (e.g., human GI). In particular, the adjustment parameters are obtained by a stepwise selective optimization process that employs a curve-fitting algorithm that estimates the change required in a value assigned to an initial absorption parameter of a developmental physiological model in order to change an output variable corresponding to the simulated rate, extent and/or concentration of a test sample at a selected site of administration for a mammalian system of interest. The curve-fitting algorithm can be regression- or stochastic-based. For example, linear or non-linear regression may be employed for curve fitting, where non-linear regression is preferred. Stepwise optimization of adjustment parameters preferably utilizes a concurrent approach in which a combination of *in vivo* pharmacokinetic data and *in vitro* data for a diverse set of compounds are utilized simultaneously for fitting with the model. A few parameters of the developmental physiological model are adjusted at a time in a stepwise or sequential selection approach until the simulated absorption profiles generated by the physiological model for each of the training/validation compounds provides a good fit to empirically derived *in vivo* data. An example of this approach is depicted in **Figures 10** and **26**. Utilization of adjustment parameters permits predictability of diverse data sets, where predictability ranges from a regression coefficient (r^2) of greater than 0.40, 0.45, 0.50, 0.55, 0.60, 0.65, 0.60, 0.65, 0.70, or 0.75 for 80% of compounds in a compound test set having a diverse range of dose requirements and a diverse range of permeability, solubility and transport mechanisms. The preferred predictability ranges from a regression coefficient (r^2) of greater than 0.60, with a regression coefficient (r^2) of greater than 0.75 being more preferred, and greater than 0.80 being most preferred. Adjustment parameters utilized for *in vivo* to *in vitro* prediction (e.g. dog to human) employs the same basic approach.

The regional correlation parameters of the PK tool include constants or ranges of constants that are utilized to estimate a selected parameter value of a first segment of the mammalian system under investigation when that value is not supplied by the user. The model performs this estimation by utilizing a function/transformation algorithm (e.g., utilizing polynomial, exponential, logarithm, or any other variety of transformation approaches) in which (1) regional correlation parameter values, and

(2) one or more values for the parameter that is supplied by the user for a second segment of the mammalian system, are utilized to estimate the value for the first segment. The regional correlation parameters may be empirically derived values or adjustment parameter values for various segments of the mammalian system of interest such as for permeability. A preferred regional correlation approach employs a polynomial-based correlation. The polynomial is based on the particular parameter to be estimated. The regional correlation is performed by logic function of the model, which when activated utilizes the function/transformation algorithm to perform the estimation. The regional correlation logic function of the model is activated when a value is missing for the selected parameter. The estimated value(s) are then utilized as input variables for the particular parameter in question. The model then proceeds by employing the estimated value for subsequent simulation. Various regional correlation parameters can be used, such as permeability, solubility, dissolution rate, transport mechanism and the like. The preferred correlation parameters are for permeability. This permits the PK tool of the invention to predict absorption of a test sample from minimal input permeability values, such as when the simulation model is a GI tract simulation model and when cell-based assays are employed to provide permeability data corresponding to a given GI segment (e.g., Caco-2 cells and colon).

The above described methodology for *in vivo* prediction from *in vitro* input also is followed for *in vivo* prediction for a first species of mammal from *in vivo* input data derived from a second species of mammal.

Since the parameter values are specific for a given physiological model (e.g., GI model-parameters, Ocular model-parameters, Blood-Brain-Barrier-parameters, etc.), parameter values are chosen accordingly. These values are obtainable de novo from experiments or from the literature, and adjustment parameters and regional correlation parameters derivable therefrom. The preferred values are based on a diverse collection of training/validation compounds for which *in vivo* pharmacokinetic data is available.

The various physiological models also may reside in a database, in part or in whole, and may be provided in the database with or without the initial parameter values. The database will preferably provide the differential equations of the model in a compartment-flow data structure that is readily portable as well as executable by the simulation engine.

An integrated physiological model corresponding to the GI tract of a mammal constructed using STELLA® and the above-described methodology is illustrated in **Figures 24-25**, and **29-39**. An example of information provided by the database is illustrated in **Appendix 4** for the gastrointestinal model depicted in **Figures 24-25** and **29-39**.

A physiologic-based simulation model of the PK tool and method of the invention may optionally include a training/validation model. This aspect of the invention can be used for determining whether the model is specific and accurate with respect to compounds of known membrane transport mechanism (e.g., passive transcellular, passive paracellular, transporter involved for absorption and secretion) and/or with respect to known drug solubility/dissolution rate limitations.

A validation model can be linked to the physiological model of the invention as illustrated in **Figure 11**. The linked system is then run to access the specificity and accuracy computed values for rate and extent of absorption. These values are then compared to empirically measured plasma values. If computed values fall outside of an acceptable range the model can be reevaluated for these compounds and adjustments made to the model.

Data Acquisition:

Input data utilized to generate an absorption profile for a test sample include permeability and solubility parameters, and optionally transport mechanism and dissolution parameters. Input data can be generated de novo following any number of techniques, or obtained from public or existing sources where available. The input data can be derived from chemical, and/or biological assays as well as theoretical predictions. By way of example, the *in vitro* assays may employ artificial (synthetic) or naturally occurring biological preparations. This includes chemical, cell and/or tissue preparations. Assays for generating input data involve screening a plurality of test samples containing isolated compounds and/or isolated mixtures of compounds per test sample in an assay characterized by measurement of (1) permeability and optionally transport mechanism for a test sample; and (2) solubility and optionally dissolution for a test sample. Methods and materials for performing the assays are based on the selected route of administration, the associated barrier(s) to absorption and proposed sampling site(s). For instance, if oral delivery is proposed for simulation and an initial sampling site is selected to be the portal vein (so as to isolate gastrointestinal absorption events from hepatic metabolism) then the input data is collected from an *in vitro* assay that best approximates the luminal barrier and segmental physiology of the gastrointestinal tract.

Examples of some common cell and tissue sources for permeability and transport mechanism assays for a selected route of administration are provided below in **Table 1**.

Table 1: Permeability and Transport Mechanism.

Route/Tissue	Cell Culture
Oral/Intestinal	Caco-2 cells HT-29 cells T84 cells Intestinal epithelial cells (IEC) SV40 T Immortalized cells Organ culture/co-culture Primary culture
Inhalation/Nasal	SV40 T immortalized cells Primary culture
Ocular/Corneal	RCE1 cells Primary cultures SV40 T immortalized cells
Oral-Buccal/Cheek	Primary cultures
Topical/Transdermal	HaCat cells Primary/co-cultures
IV/Hepatic	Hepatic carcinoma cell lines Primary cultures Co-cultures SV40 T immortalized cells
IV/Blood Brain Barrier	Primary culture SV40 immortalized cells

Examples of some common parameters for solubility and dissolution assays for a given route of administration are provided below in **Table 2.**

Table 2: Solubility and Dissolution Parameters.

Route/Anatomy/Physiology		***In vitro* Parameters**
Oral	Gastrointestinal (GI) tract • Stomach • Duodenum • Jejunum • Ileum • Colon	• pH • Temperature • Concentration of test sample • Volume • Osmotic pressure • Admixing conditions • Physiologic Fluid/Buffer/solvent system • Excipients • Other Additives • Test chamber composition
Buccal/Sublingual	Mouth • Cheek • Tongue	
Rectal	Lower GI tract • Colon • Rectum	
Parenteral	Skin Muscles Veins	
Aerosol	Respiratory system • Nose • Lungs • Mouth	
Transdermal	Skin • Topical • Ear	

In vitro and *in vivo* techniques for collecting permeability and transport mechanism data using cell- and/or tissue-based preparation assays are well known in the art (Stewart et al., Pharm. Res. (1995) 12:693-699; Andus et al., Pharm. Res. (1990) 435-451; Minth et al., Eur. J. Cell. Biol. (1992) 57:132-137; Chan et al., DDT 1(11):461-473). For instance, *in vitro* assays characterizing permeability and transport mechanisms include *in vitro* cell-based diffusion experiments and immobilized membrane assays, as well as *in situ* perfusion assays, intestinal ring assays, intubation assays in rodents, rabbits, dogs, non-human primates and the like, assays of brush border membrane vesicles, and everted intestinal sacs or tissue section assays. *In vivo* assays for collecting permeability and transport mechanism data typically are conducted in animal models such as mouse, rat, rabbit, hamster, dog, and monkey to characterize bioavailability of a compound of interest, including distribution, metabolism, elimination and toxicity. For high-throughput screening, cell culture-based *in vitro* assays are preferred. For high-resolution screening and validation, tissue-based *in vitro* and/or mammal-based *in vivo* data are preferred.

Cell culture models are preferred for high-throughput screening, as they allow experiments to be conducted with relatively small amounts of a test sample while maximizing surface area and can be utilized to perform large numbers of experiments on multiple samples simultaneously. Cell models also require fewer experiments since there is no animal variability. An array of different cell lines also can be used to systematically collect complementary input data related to a series of transport barriers (passive paracellular, active paracellular, carrier-mediated influx, carrier-mediated efflux) and metabolic barriers (protease, esterase, cytochrome P450, conjugation enzymes).

Cells and tissue preparations employed in the assays can be obtained from repositories, or from any higher eukaryote, such as rabbit, mouse, rat, dog, cat, monkey, bovine, ovine, porcine, equine, humans and the like. A tissue sample can be derived from any region of the body, taking into consideration ethical issues. The tissue sample can then be adapted or attached to various support devices depending on the intended assay. Alternatively, cells can be cultivated from tissue. This generally involves obtaining a biopsy sample from a target tissue followed by culturing of cells from the biopsy. Cells and tissue also may be derived from sources that have been genetically manipulated, such as by recombinant DNA techniques, that express a desired protein or combination of proteins relevant to a given screening assay. Artificially engineered tissues also can be employed, such as those made using artificial scaffolds/matrices and tissue growth regulators to direct three-dimensional growth and development of cells used to inoculate the scaffolds/matrices.

Epithelial and endothelial cells and tissues that comprise them are employed to assess barriers related to internal and external surfaces of the body. For example, epithelial cells can be obtained for the intestine, lungs, cornea, esophagus, gonads, nasal cavity and the like. Endothelial cells can be obtained from layers that line the blood brain barrier, as well as cavities of the heart and of the blood and lymph vessels, and the serious cavities of the body, originating from the mesoderm.

One of ordinary skill in the art will recognize that cells and tissues can be obtained de novo from a sample of interest, or from existing sources. Public sources include cell and cell line repositories such as the American Type Culture Collection (ATCC), the Belgian Culture Collections of Microorganisms (BCCM), or the German Collection of Microorganisms and Cell Cultures (DSM), among many others. The cells can be cultivated by standard techniques known in the art.

Preferred assays for collecting permeability data utilize devices and methods that measure change in resistance or conductivity of a membrane system by ion flux. Any device suitable for such studies can be employed. These include voltage-clamp type devices and methods that employ either cell cultures or precision tissue slices. Diffusion chamber systems utilizing cultured cells grown on permeable supports to measure permeability are preferred. More preferred devices are readily adapted for high-throughput and automated screening. Examples of such devices are known and exemplified in U.S. Patent No. 5,599,688; WO 96/13721; and WO 97/16717. These devices also can be adapted for examining transport mechanisms. As can be appreciated, however, measurement of resistance, conductivity and/or ion flux is not required to determine permeability of compounds. Many other techniques are available and can be employed in the invention. For instance, permeability data also may be predicted using theoretical models to approximate this parameter, for example, from SAR/QSAR (e.g., log P, molecular weight, H-bonding, surface properties).

Transport mechanism of a test sample of interest can be determined using cell cultures and/or tissue sections following standard techniques. These assays typically involve contacting cells or tissue with a compound of interest and measuring uptake into the cells, or competing for uptake, compared to a known transport-specific substrate. These experiments can be performed at short incubation times, so that kinetic parameters can be measured that will accurately characterize the transporter systems, and minimize the effects of non-saturating passive functions. (Bailey et al., Advanced Drug Delivery Reviews (1996) 22:85-103); Hidalgo et al., Advanced Drug Delivery Reviews (1996) 22:53-66; Andus et al., Pharm. Res. (1990) 7(5):435-451).

For high-throughput analyses, cell suspensions can be employed utilizing an automated method that measures gain or loss of radioactivity or fluorescence and the like such as described in WO 97/49987.

In a preferred embodiment, transport mechanism is determined using high-throughout transporter screening cell lines and assays. In this aspect of the invention a cell line is selected and/or manipulated to over-express one or more transporter proteins, and/or enzymes. The cells are then used to rapidly identify the mechanism(s) by which a compound is transported across the physiological barrier of interest. Transporters of interest represent the basic categories of transport including uptake and efflux transporters. These transporters aid in the movement of materials in biological systems, into and out of cells and across cellular layers. Natural combination(s) of enzyme(s) and transporter(s) also can provide the basis of a high-throughput transport mechanism screening assay. For instance, certain enzymes or transporters require secondary enzymes or transporters to function in a normal physiological mode, i.e., cytochrome P4503A is co-regulated with P-glycoprotein. These proteins share the same substrate and their genes are co-regulated. Thus multiple artificial combination(s) of transporter(s) and enzyme(s) can be employed for characterizing transport mechanism of a test sample of interest. Examples of possible combinations of a transporter and/or enzyme in a host cell of interest include cell-transporter-enzyme, cell-transporter, cell-enzyme, cell-enzyme-enzyme, and cell-transporter-transporter. Examples of transporters that can be used to transfect the host cell of interest include peptide transporters (PepT1), amino acid transporters, organic cation transporters (OCT1), organic anion transporters, nucleotide transporters (N1, N2, N3, ES, EI), glucose transporters (SGLT1, GLUT 1 through GLUT 7), monocarboxylate transporters (MCT1), and multi-drug transporters (LRP, MDR, MRP, PGP). Examples of enzymes that can be used to transfect the host cell are Phase I and II enzymes, cytochrome P450, 3A, 2D and the like.

Nucleic acid and/or amino acid sequences for transporters/enzymes can be identified in various genomic and protein related databases. Examples of publicly accessible databases include GenBank (Benson et al., *Nucleic Acids Res* (1998)26(1):1-7; USA National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, Bethesda, MD, USA), TIGR

Database (The Institute for Genomic Research, Rockville, MD, USA), Protein Data Bank (Brookhaven National Laboratory, USA), and the ExPASy and Swiss-Protein database (Swiss Institute of Bioinformatics, Genève, Switzerland).

Any number of known techniques can be used to prepare nucleic acid encoding a transporter(s) and/or enzyme(s) of interest. To express a target protein in a host cell the nucleotide sequence coding for the polypeptide is inserted into an appropriate expression vector, i.e., a vector that contains the necessary elements for the transcription and translation of the inserted coding sequence. The host cell line can be stably or transiently transfected by methods known in the art. Examples of transient transfection methods include calcium phosphate, electroploration, lipofectamine, and DEAE dextran. A cell line can be stably transfected using methods known in the art such as calcium phosphate. In addition, the host cell can be infected with a retrovirus containing a target protein of interest, resulting in stable expression of the desired target protein. Host cells that express the target gene product can be identified by standard techniques. These include, but are not limited to, detection of the protein as measured by immunoprecipitation and Western blot analysis or by measuring a specific biological response.

For synthesis in a cell, a target transporter/enzyme protein can be generated by standard techniques. Cells that naturally express a target protein can be employed. Transfection and transformation of a host cell with DNA encoding a protein of interest also can be used. For example, a polymerase chain reaction (PCR) based strategy may be used to clone a target DNA sequence encoding all or part of a target membrane polypeptide of interest. (See, e.g., "PCR Cloning Protocols: From Molecular Cloning to Genetic Engineering," B.A. White, ed., Humana Press, Methods in Molecular Biology, Vol. 67, 1997). For example, PCR can be used for cloning through differential and subtractive approaches to cDNA analysis, performing and optimizing long-distance PCR, cloning unknown neighboring DNA, and using PCR to create and screen libraries. PCR also can be used to introduce site-specific and random mutations into DNA encoding a target protein of interest.

For general cloning purposes, complementary and/or degenerate oligonucleotides corresponding to conserved motifs of the target membrane polypeptide may be designed to serve as primers in a cDNA and/or PCR reaction. Templates for primer design can be obtained from any number of sources. For example, sequences, including expressed sequence tags (ESTs) can be obtained from various databases, such as GenBank, TIGR, ExPASy and Swiss-Protein databanks. Homology comparisons performed using any one of a number of alignment readily available programs that employ search engines to find the best primers in a sequence based on various algorithms. Any number of commercially available sequence analysis packages, such as Lasergene, GeneWorks, DNASIS, Gene Jockey II, Gene Construction Kit, MacPlasmap, Plasmid ARTIST, Protein Predictor, DNA/RNA Builder, and Quanta. (See, e.g., "Sequence Data Analysis Guidebook," Simon R. Swindell, ed., Humana Press, 1996). The information can be used to design degenerate primers, nested/multiplex primers, site-directed mutagenesis, restriction enzyme sites etc. Primers can be designed from homology information, and computer programs can be used for primer design as well. Examples include "Primer Premier 4.0" for automatic primer selection (CloneTech, Inc.). The amplified cDNA and/or PCR fragment may be used to isolate full-length clones by radioactive or non-radioactive labeling of the amplified fragment and screening a library.

Alternatively, transporter/enzyme DNA cloned from one source may be utilized to obtain a corresponding DNA sequence from other sources. Specifically, a genomic and/or cDNA library constructed from DNA and/or RNA prepared from a cell known or expected to express the target transporter/enzyme may be used to transform a eukaryotic or prokaryotic host cell that is deficient in the putative gene. Transformation of a recombinant plasmid coding for the protein into a deficient host cell would be expected to provide the cell with a complement product corresponding to the protein of interest. In some cases, a host cell can be selected to express a particular phenotype associated with the target polypeptide and thus may be selected by this property. For a review of cloning strategies which may be used, see e.g., Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual, Cold Springs Harbor Press, New York; and Ausubel et al., 1989, Current Protocols in Molecular Biology, Green Publishing Associates and Wiley Interscience, New York.

To express a target transporter/enzyme in a host cell the nucleotide sequence coding for the protein, or a functional equivalent for modular assembly as described above, is inserted into an appropriate expression vector, i.e., a vector which contains the necessary elements for the transcription and translation of the inserted coding sequence. Host cells containing the coding sequence and that express the target gene product may be identified by standard techniques. For example, these include but are not limited to DNA-DNA or DNA-RNA hybridization; the presence or absence of "marker" gene functions; assessing the level of transcription as measured by the expression of mRNA transcripts in the host cell; and detection of the gene product as measured by immunoassay or by its biological activity.

Once a clone producing the target transporter/enzyme is identified, the clone may be expanded and used to over express the protein(s). If desired, the proteins may be purified using techniques well-known in the art including, but not limited to immunoaffinity purification, chromatographic methods including high performance liquid chromatography or cation exchange chromatography, affinity chromatography based on affinity of the polypeptide for a particular ligand, immunoaffinity purification using antibodies and the like. The purified proteins can then be bound to an artificial membrane matrix and utilized for assessing interaction of compounds to the transporter/enzyme of interest.

Some commonly used host cell systems for expression of transport proteins and enzymes include *E. coli*, Xenopus oocytes, baculovirus, vaccinia, and yeast, as well as many higher eukaryotes including transgenic cells in culture and in whole animals and plants. (See, e.g., G.W. Gould, "Membrane Protein Expression Systems: A User's Guide," Portland Press, 1994, Rocky S. Tuan, ed.; and "Recombinant Gene Expression Protocols," Humana Press, 1996). For example, yeast expression systems are well known and can be used to express and recover target transporter/enzyme systems of interest following standard protocols. (See, e.g., Nekrasova et al, *Eur. J. Biochem.* (1996) *238*:28-37; Gene Expression Technology Methods in Enzymology 185: (1990); Molecular Biology and Genetic Engineering of Yeasts CRC Press, Inc. (1992); Herescovics et al., FASEB (1993) 7:540-550; Larriba, G. *Yeast* (1993) *9*:441-463; Buckholz, R.G., *Curr Opinion Biotech* (1993) *4*:538-542; Mackett, M, "Expression of Membrane Proteins in Yeast Membrane Protein Expression Systems: A Users Guide," pp. 177-218, Portland Press, (1995).

For high-resolution screening and validation, tissue-based assays may be employed to characterize transport mechanisms. For example, of the cytochrome P450 superfamily, CYP3A enzymes represent the most abundant isoforms in the liver and they are responsible for the metabolism of compounds of diverse chemical structure. The uptake of a compound into hepatocytes can be mediated by passive or carrier processes. Once in the parenchymal cell of the liver, the drug can be metabolized or bind to intracellular proteins. The drug or its metabolite(s) may return to the circulation or exit from the hepatocyte into the bile canaliculus, again by passive or carrier-mediated transport, before secretion in bile. Experimental systems have been devised to study these processes in isolation. Examples of such systems include isolated perfused rat liver (IPRL), and bile duct cannulated (BDC) rat models. (Chan et al., DDT (1996) 1:461-473).

Tissue from transgenic animals designed to express particular transport properties in one or more particular tissues also may be utilized to characterize transport mechanisms. In this aspect of the invention, an animal can be genetically manipulated to express or not express one or more specific proteins in a tissue of interest, e.g. transporter protein in duodenum tissue. Tissue from the genetically engineered animal can then be used to examine transport mechanisms in a tissue-based assay. Transgenic animal methodologies are well known (Gordon et al., Hum. Cell (1993) 6(3):161-169; and Jaenisch, R., Science (1998) 240:1468-1474).

Artificially engineered tissue also can be used for permeability assays, such as tissues generated *ex vivo* for use as skin grafts, transplants, and the like. Such tissues can be obtained using standard techniques. See, for example, U.S. Patent Nos. 5,759,830; 5,770,193; and 5,770,417.

Solubility and dissolution data can be obtained in an *in vitro* assay by testing each sample of interest in an appropriate physiologic fluid/buffer system that best approximates the particular physiological system selected as the barrier to absorption. A solubility profile is a plot of solubility of a test sample at various physiological conditions. As an example, the natural pH environment of the gastrointestinal tract varies from acidic in the stomach to slightly alkaline in the small intestine and fluid composition for each segment may vary as well. The solubility profile provides an estimation of the completeness of dissolution of a test sample in a particular physiological compartment or anatomical entity. In this instance, a panel of test wells each having different pHs and physiological fluid composition can be employed to generate a solubility profile for each test sample. Solubility and dissolution data can also be predicted using theoretical models to approximate these values, for example, from SAR/QSAR information.

In vitro dissolution assays measure the rate and extent of dissolution of a test sample in an aqueous solution. Various parameters are considered when performing a dissolution assay and are well known in the art. These parameters include size of the experimental vessel, amount of agitation and nature of the stirrer, temperature and nature of the dissolution medium, pH, viscosity, and design of the dissolution apparatus. Standard methods known in the art for measuring dissolution include rotating basket, paddle, rotating bottle, flow-through dissolution, intrinsic dissolution, and peristalsis methods. These methods can be adapted and used as a guide for high-throughput solubility and dissolution testing.

For high-throughput collection of solubility and dissolution data, automated methods of solid and liquid handling are employed. This method involves addition of samples to a multi-well or multi-tube/plate system. The data associated with these tubes/plates, such as physiologic fluid/buffer system, volume, concentration, pH and tube/plate maps, is transferred into an inventory system. The inventory system generates codes containing updated information pertaining to the aliquoting, diluting, or pooling methods applied to the original tubes/plates. Tasks created in the database are then carried out physically in coded tubes/plates. Aliquots are then distributed to designated screen sites. After testing, the solubility profiles are generated and ported to a database for access and analysis.

Properties in addition to absorption that can be utilized as input into the PK tool and method of the invention when adapted with the appropriate compartments include metabolism, distribution, and elimination, and optionally toxicity. As with absorption, assays to characterize the relevant data are based on the selected route of administration. Metabolism or biotransformation refers to the biochemical transformation of a compound to another chemical form. The biotransformation process typically results in a metabolite that is more polar (water-soluble) than the original parent molecule.

Most tissues have some metabolizing capacity but the liver is by far the most important organ, on the basis of size if not always concentration of target compound metabolizing enzyme. Phase I reactions are defined as those that introduce a functional group to the molecule and phase II reactions are those that conjugate those function groups with endogenous moieties.

Since metabolism is a drug clearance process, metabolism of a compound contributes to elimination of the compound. Thus, compounds can be tested for metabolism in order to generate input data that considers disposition of a test compound after or concurrent with administration using standard techniques known in the art. (See, e.g., Sakuma & Kamataki, Drug metabolism research in the development of innovative drugs, In: Drug News & Perspectives (1994) 7 (2):82-86).

Metabolism assays for high-throughput screening preferably are cell-based (cells and cellular preparations), whereas high resolution screening can employ both cell and tissue-based assays. In particular, test samples from compound libraries can be screened in cell and tissue preparations derived from various species and organs. Although liver is the most frequently used source of cells and tissue, other human and non-human organs, including kidney, skin, intestines, lung, and blood, are available and can be used to assess extra-hepatic metabolism. Examples of cell and tissue preparations include subcellular fractions (e.g., liver S9 and microsomes), hepatocytes (e.g., collagenase perfusion, suspended, cultured), renal proximal tubules and papillary cells, re-aggregate brain cells, bone marrow cell cultures, blood cells, cardiomyocytes, and established cell lines as well as precision-cut tissue slices.

Examples of *in vitro* metabolism assays suitable for high-throughput screening include assays characterized by cytochrome P450 form-specific metabolism. These involve assaying a test compound by P450 induction and/or competition studies with form-specific competing substrates (e.g., P450 inhibitors), such as P450 enzymes CYP1A, 3A, 2A6, 2C9, 2C19, 2D6, and 2E1. Cells expressing single or combinations of these or other metabolizing enzymes also may be used alone or in combination with cell-based permeability assays. A high-throughput cell-based metabolism assay can include cytochrome P450 induction screens, other metabolism marker enzymes and the like, such as with measurement of DNA or protein levels.

Suitable cells for metabolism assays include hepatocytes in primary culture. Computer-implemented systems for predicting metabolism also may be employed.

For distribution and elimination data, *in vitro* assays can be performed to assess protein binding to a test compound, since protein binding can affect compound distribution and elimination. In general, it is free compound that diffuses into cells and tissues. Binding can be classified as restrictive or permissive with regard to elimination, or quantitatively defined in terms of affinity. Affinity of the binding is defined as low or high when reversible, or more unusually when irreversible binding occurs. The biological half-life of a test compound will increase due to its interaction with a protein. Usually, the higher the affinity the lower the elimination that may be observed. Albumin is by far the most frequent contributors to plasma protein binding since it comprises about one half of the total plasma proteins. The a1-Acid glycoprotein also plays an important role in the protein binding of a compound since it has an affinity for bases (many drugs are weak bases). It is an acute phase reactant and its concentration rises in inflammatory processes, malignant disease and stress. Lipoproteins (HDL, LDL or VLDL) bind drugs that are highly liposoluble and a fairly specific ligand-protein interaction occurs between certain steroids and gamma globulins. Thus, *in vitro* protein binding assays that employ one or more of albumin, a1-acid glycoprotein, lipoprotein, steroid and gamma globulins may be utilized to collect distribution and elimination data that can be utilized for further data collection.

Similarly, toxicity of a test compound may also be assayed and used to generate relevant toxicity data for a test compund. Any number of techniques in the art may be employed for this purpose. Preferred methods are *in vitro*. Examples include determination of toxicity mechanisms, determination of cytotoxic potentials in cell and tissues of target organs, estimation of therapeutic indices from *in vitro* data, cytotoxicity screening of closely related drug compounds in cells from the same mammal or from different species, detection and quantification of peroxisome proliferation, screening of agents to prevent or reverse cytotoxicity, and specialized studies on target cells using co-incubation systems, e.g., red blood cells and hepatocytes.

Toxicity assays may utilize any technique that provides a toxicity parameter as an endpoint. For high-throughput screening, cell based assays are preferred. This includes gene expression (e.g., protein or nucleic acid based) enzymatic activity, and morphology screens and the like. Examples of cell-based assays include *in vitro* peroxisome proliferation studies, which can be used to assay palmitoyl CoA-oxidation in primary hepatocyte culture, with or without concurrent measurement of DNA or protein levels. Cytotoxicity assays in primary cultures also can be utilized, and include screening for cytotoxicity in hepatocytes or renal proximal tubules, enzyme release (lactate dehydrogenase), and MTT conversion (mitochondrial function) following standard techniques. Computer-implemented SAR/QSAR models for predicting toxicity also may be employed, such as when structural information is available.

PK Tool and System Structure:

The PK tool and system of the invention has the structure shown in **Figure 4**. The I/O system provides the user's inputs to the simulation model of the mammalian system of interest. The simulation engine in turn computes one or more of the bioavailability parameters of the compound in the context of one or more physiologic-based segments of the mammalian system under investigation. The output of the simulation engine is then provided to the I/O system.

Operations of the PK tool and system are exemplified in **Figures 3** and **44-46**. After start, the first block is the I/O block (1), where the user enters the inputs and outputs to the system. The I/O system includes I/O panels, for example graphical user interfaces. This may include sub-panels depending on the selected model (see, e.g. **Figure 47**). The I/O system may optionally include one or more databases of simulation models and/or parameters for a given simulation model that the user may access as illustrated in **Figure 45**. The PK tool and system starts with the user inputs and then computes and displays the results in the output space. The input and output space can be selected, e.g., by toggling, or by a menu. It is to be understood that on-line helps also are available to give a user information, and to guide the user through the PK tool and system user interface.

For input, the Menu function presents various choices to the user. These choices include dose, permeability, and solubility among others. The user then enters the relevant values corresponding to a given physiological segment of the selected mammalian system in question. Depending on the simulation model that the user chooses, the Menu function will provide options for data input, such as pH, transit time, run time, and formulation release rate.

The Menu function also presents various choices to the user after the results for a simulation have been obtained. The choices open to the user include one or more of the functions “Rate of Absorption,” “Extent of Absorption,” “Concentration,” “Print Graph,” “Print Table,” and “Quit” among others.

For predicting absorption parameters, input of the data is the first operation that the PK tool and system of the invention performs when activated. In this operation, the user enters the appropriate value of each input variable into the input panel in a form readable or convertible by the system to a readable form and obtains complete results in the output panel. Alternatively, the PK tool and system can be adapted to receive structural information that the system, or a separate interfaced system converts to the relevant input parameter values. For this function the user inputs the compound structure in a form readable or convertible by the system to a readable form. This includes standard chemical formulas, chemical names, SMILE strings, as well as two-dimensional and/or three-dimensional structures.

After the user inputs the initial data, the Start Simulation function is selected. In the simulation function, the simulation engine is activated. The user may then choose to invoke the Stop Simulation function, to terminate the simulation, or allow the simulation engine to proceed with until a user specified or system default time point is reached. The user may then view, print, save and/or export then results using output functions, including printing of the I/O panel. This includes numerical, tabular, and graphical formats. These options are selected by the user through the Menu function.

The Quit function exits the PK tool and system. One aspect of the output functions and the Quit function is to save the generated information in a format that allows them to be an input to other programs, such as the SAR or QSAR CAD program.

Forward Mode Operation of the PK Tool:

In the forward mode operation mode, the user enters the input data, and the PK tool reacts as described above. In one embodiment, the PK tool displays a numerical representation or graphic of the test compound or selected PK profile thereof. Also displayed are parameters that can effect fate of the compound in one or more compartments of the mammal, e.g., the dose, formulation, pH, fluid volume, fluid absorption (fluid secretion), dissolution rate, cumulative dissolution, transit, pH-dependent solubility and dissolution and the like. Other variables may also be available, e.g., through a data box.

The forward mode operation of the simulation engine displays the resulting PK parameters, such as absorption. Changing any parameter causes recalculation of the PK quantities, invoking the the simulation engine and its associated simulation model. The forward mode operation provides either, or both, a display or a printout of the PK parameters for a test compound.

Backward Mode Operation of the PK Tool:

In backward mode operation of the PK tool, the user is allowed to assess formulations for a compound. In this aspect of the invention, the user specifies the required absorption profile, or absorption parameter for a compound. The tool then generates the formulation release rates for the compound that meets the requirements. The user can then compare the solution set, against previously qualified compounds and formulation designs drawn from a database and new, unqualified designs created by the tool and method of the invention.

Predictability:

The PK tool and method of the invention permit a high level of accuracy in predicting bioavailability of molecules from the following four classes of compounds: a) passive transcellular; b) passive paracellular; c) transcellular transporter involved; d) apically recycled. The evaluation is based on the difference between bioavailability values predicted by the model and known bioavailability values. For example, conformation of predictability for human GI absorption values for passive transcellular molecules is evaluated with dissolution rate limitations and solubility limitations. If the computed values fall outside of an acceptable range ($r^2 > 0.75$ predictability), the PK model is reevaluated for these compounds and adjustments made to the model. Similarly, absorption measures that deviate from known values are reevaluated and appropriate modifications made to the model (e.g. iterative process).

The PK model can be used to predict bioavailability in a mammal using dose (actual or estimated) and various input data. Examples include (1) permeability data alone; (2) permeability data together with solubility and dissolution data; (3) permeability data together with animal data; and/or (4) permeability, animal and human clinical data. Validation of the model is defined as follows, where greater than 80% of the compounds tested will fall within the following prediction criteria.

1. Predictability of the PK tool using permeability data alone with limits for dose and elimination rate ($r^2 > 0.75$ predictability).

2. Predictability of the PK tool using permeability and solubility data along with limits for dose and elimination rate ($r^2 > 0.75$ predictability).

3. Predictability of the PK tool using permeability data together with animal data for pharmacokinetics together with limits for dose ($r^2 > 0.85$ predictability).

4. Predictability of the PK tool using permeability and animal or human IV data to predict absorption values for molecules with solubility limitations ($r^2 > 0.85$ predictability).

The correlation coefficient can be calculated using data from the predicted line from pharmacokinetic fitting as the observed data points and as the predicted fit, and the output of physiologic-based simulation model coupled to the systemic kinetics for that compound. The prediction power of a given physiological simulation model can be demonstrated by simulating the plasma levels in compounds. Other methods can be utilized to assess the predictive power of the model to achieve the same end result (i.e., evaluation of model performance).

The method and PK tool of the invention allows the drug developer to go from a set of user inputs, to predicting the fate of the compound in a mammalian system of interest, to selection of a compound design input to a SAR or QSAR CAD tool, and to chemical synthesis development, validation and high level drug development. The PK tool and system may advantageously be interfaced with other databases and/or systems. For example, the system may be built around an expert system-database manager path. The menu can invoke the on-line documentation, the database, and any member of the expert system-database system.

The PK tool and method of the invention can be used to predict the rate and extent of absorption of compounds as well as regional concentrations relative to one or more selected sampling sites across a physiological barrier of a mammalian system of interest. The PK tool and method of the invention also can be used in combination with prediction of additional bioavailability parameters such as distribution, metabolism and elimination, as well as toxicity. Thus this information can be used to supplement and significantly reduce animal testing during pre-clinical testing. The PK tool and method of the invention also are particularly useful for screening compounds earlier in the drug discovery process. For instance, the PK tool and method may be employed in the screening and ranking of compounds before, during and/or after receptor activity testing, thus increasing the odds of selecting a lead compound that will survive clinical studies, resulting in decreased development costs, faster approval time, and consequent lower drug prices. This permits selection and ranking of lead compounds that not only have optimal receptor activity, but also exhibit optimal bioavailability.

The following Examples are intended to illustrate various aspects of the invention and are not intended to limit the scope of the invention.

EXAMPLES

Example 1: Introduction to Model Design and Development

A physiologic-based simulation model for predicting oral absorption of a compound in a mammal from *in vitro* (e.g., tissue, cell and SAR/QSAR) and *in vivo* data (e.g., human) was constructed in two primary stages. The first stage involved development of a mass-based multi-compartment simulation model (mass model), a volume-based multi-compartment simulation model (volume model) and an integrated mass-volume multi-compartment simulation model (mass-volume model). These models were individually tested and validated for five segments of the GI tract: the stomach, the duodenum, the jejunum, the ileum, and the colon. The second stage involved development of an integrated multi-compartment physiological model of the GI tract (GI model). The models were developed using a combination of *in vitro* data and *in vivo* data.

A computer-based mathematical model development tool with a graphical user interface was employed to design and construct the initial simulation models. The computer program STELLA® was selected as suitable for this purpose since it permitted compartment model building and mathematical equation modification and at each stage of the build, as well as calculation of flow between compartments at user-specified time intervals (dt) with user-specified input functions and values. An example of iconic tools and description, as well as graphically depicted compartment-flow models generated using STELLA® and their relation to a conventional pharmacokinetic IV model is illustrated in **Figures 5-8**.

Example 2: Compound Data Sets

Compound data sets for development, and thus building, testing, training and validation of the models were obtained from various sources including the literature and cell, tissue, animal and human tests as described herein. The data sets included relevant physiological parameters related to absorption of a compound including GI tract related parameters (e.g., pH, initial volumes, surface area, average transit time, volume transfer rates, new water absorption etc.) and physicochemical compound related parameters (e.g., dissolution, permeability, solubility etc.).

Data sets were selected for compounds that permitted development and isolated testing and validation for each stage of the build. Compounds suitable for this purpose were chosen as follows. For the mass, volume and integrated mass-volume simulation models, a candidate compound was chosen based on the premise that the best candidate compound for model development would not be a drug that is highly correlated pharmacokinetically between cell, tissue, animal and humans, but one that is poorly correlated. That is, a compound predicted to have high total absorption in humans based on pre-clinical studies, but ultimately exhibited poor absorption in humans when tested in clinical trials was chosen. Additionally, a compound was selected that is not subject to pre-absorptive or hepatic metabolism so as to isolate absorption components of the models from pre-absorptive and metabolic factors. Gancyclovir (9-(1,3-dihydroxy-2-propoxymethyl)guanine, monosodium salt (DHPG) or Cytovene) was suitable for this purpose. Also, significant animal and human clinical data was publicly available for Gancyclovir (Jacobson *et al.*, *Antimicrobial Agents and Chemotherapy*, Vol. 31, No. 8, p. 1251-1254 (1987); New Drug Application for Gancyclovir Sodium (Syntex, Inc. USA), obtained from the Food & Drug Administration; Drew *et al.*, *New England Journal of Medicine*, (1995) 333:615-610; and Anderson *et al.*, *Clinical Therapeutics*, (1995) 17:425-432 (1995)).

For development and testing of the integrated GI model, a set of training and testing lead drug compounds in various stages of human clinical testing were selected. This test set included compounds having diverse dosage requirements and ranges of permeability, solubility, dissolution and transport mechanisms, as shown below in **Table 3**.

Table 3.

Compound Test Set				
Compound	Permeability	Solubility	Dose	Mechanism of Absorption
α1	++++	++++	+++++	active
α2	++	+++	+++++	paracellular
α3	+	+	++++	unclassified
α4	+	++++	++	transcellular
α5	+	+++	++++	paracellular
α6	++++	++	++++	transcellular
α10	++++	+++++	+	transcellular
β1	+++++	+++++	+	transcellular
β2	++++	++	++	transcellular
β3	+	+	+++	paracellular
β5	++++	++	+++	unclassified
β6	+	+++++	+++	unclassified

+++++ = greatest value & + = lowest value

Example 3: Experimental Data Collection and Processing

Experimentally derived *in vivo* and *in vitro* data was obtained as follows: To ensure quality data was used for training and validation, experimental conditions were specific enough to ensure proper data collection techniques, but flexible to allow minor and insignificant variations in individual protocols. Data sets used for model development included individual data points, i.e., raw data, that was analyzed and processed by stepwise regression analysis using a least squares minimization technique or similar fitting tool. In particular, data processing for permeability involved separation of compounds by absorption mechanism and into training and validation sets. pH dependent solubility profiles were interpolated to obtain complete profiles. For dissolution, data points were fit to determine dissolution rates. For human clinical data, data analysis and processing employed a pharmacokinetic IV/PO model and weighted least-squares regression analysis (See **Figure 18**). The IV/PO model includes a central compartment in equilibrium with a peripheral compartment, a pre-systemic compartment re-circulated with the central compartment and for input

PO doses (error function input), a hepatic compartment, as well as an IV dose and first-order elimination compartment. The plasma sample is taken from the central compartment, and the FDp sample from the hepatic compartment.

A. Human *In vivo* Data – Oral (PO)

Plasma levels following oral administration (PO) in humans were used to determine the amount of compound input to the hepatic vein (FDp) as a function of time. Plasma levels of drug in humans following oral administration of drug solution or suspension after an overnight fast were used as a data set. If no solutions or suspensions were administered, formulated dosage form data were used. The PO profiles included individual data points for each patient enrolled in the study from the time of administration through 24 hours to 32 hours after administration, along with dosage. If multiple dose regimens were administered, plasma profiles for all doses were used.

B. Human *In vivo* Data – Intravenous Administration (IV)

Plasma levels following intravenous administration (IV) in humans were used to determine the amount of drug input to the hepatic vein (FDp) as a function of time. IV profiles included individual data points for each patient enrolled in the study from the time of administration through 24 hours to 32 hours after administration, along with the dose. If multiple dosage regimens were administered, plasma profiles for all doses were used.

C. *In vitro* Permeability Data

In vitro permeability data was used to calculate drug fluxes across various regions of the intestinal mucosa. This included rabbit intestinal tissue from one or more of duodenum, jejunum, ileum and colon, and Caco-2 cells. The mechanism of transport, such as passive transcellular or paracellular, carrier-mediated absorption, carrier-mediated secretion, or mixed mechanism, was determined for several test compounds and permeabilities for each mechanism and assessed as listed in **Table 4**. Protocols for permeability assays are described in **Example 4**.

Table 4: Transport mechanism permeabilities and parameters for each GI region.

Mechanism	Permeabilities	Parameters
Passive transcellular	Apical to basolateral (AP to BL)	P_e
Passive paracellular	AP to BL	P_e
Carrier-mediated absorption	AP to BL without inhibition	K_m, P_c, and P_m, or P_e at entire concentration range
Carrier-mediated secretion	AP to BL and BL to AP without inhibition	P_m, P_c, and P_m, or P_e at entire concentration range

D. Solubility Data

Solubilities of test compounds as a function of pH were determined from pH 1.5 to 8.2 in increments of 0.1 pH units. Protocols describing conditions for solubility determination are found in **Example 4**. Alternatively, solubility at each pH unit from 1.5 to 8.0 was used, with a minimum of 5 data points at pH 1.5, 6.0, 6.5, 7.0, and 7.5. These solubilities were used to calculate the amount of soluble compound available for absorption across the intestinal mucosal barrier.

E. Dissolution Data

The dissolution of test compounds as a function of pH were determined at pH 1.5, 6.0, 6.5, 7.0, and 7.5. Protocols describing conditions for dissolution determination are found **Example 4**. The dissolution of powdered compound, and alternatively, dissolution/disintegration data for the formulated dosage form used to collect oral plasma profiles were used. The dissolution data were used with solubility data to calculate the amount of drug available for absorption across the intestinal mucous within each region of the intestine.

Example 4: Protocols for Data Collection

Provided below are detailed protocols utilized for collecting and calculating data described in **Example 3**. These protocols were employed to ensure the quality of the data provided for development of the simulation models.

A. *In vitro* Permeability Protocols

1. Diffusion Chambers

Permeability data is determined using intestinal tissue in vertical diffusion chambers similar in design to NaviCyte 8X24 mm, 9mm Low-volume, or 9mm round tissue diffusion chambers. The chamber system used maintains the tissue as well as the donor and receiver buffers at 37°C. Both the donor and receiver buffers within the chamber are continuously mixed throughout the experiment.

2. Mathematical Calculations

Effective permeability (Pe) is calculated using **Equation 2.**

$$P_e = \frac{V}{AC_0} \bullet \frac{dC}{dt} \qquad \text{(Eq. 2)}$$

where V is the volume of the receiver chamber (ml), A is the surface area available for diffusion (1.78 cm2 for 8X24 mm chambers, 0.64 cm2 for 9 mm round and Low-volume chambers), C_0 is the donor concentration, and dC/dt is calculated as the slope of the regression line of the corrected receiver concentration (see Sampling) v. time plot. Two conditions must be satisfied for this equation to apply: (1) sink conditions in the receiver chamber, i.e. the accumulated concentration, must be virtually zero when compared to the donor concentration; and (2) the donor concentration must be constant (C_0) throughout the experiment.

The parameters for carrier-mediated absorption and secretion are calculated using **Equation 3.**

$$P_e = \frac{P_c}{1 + \frac{C_0}{K_m}} + P_m \qquad \text{(Eq. 3)}$$

where Pc is the carrier-mediated permeability, Pm is the passive permeability, Km is the affinity of the drug for the carrier, and C_0 is the donor concentration. Pc, Pm, and Km are calculated using non-linear regression, Pe is calculated using **Equation 2,** and C_0 is given as part of the experimental conditions. To obtain valid parameter values, Pe is determined for a sufficient number of C_0's to determine Km using **Equation 3** (a minimum of 6 C_0's is recommended ranging between the analytical limit and the solubility limit). If Pe values are provided, the variability of the mean as well as the number of experiments performed for each concentration are provided to allow accurate regression analysis.

3. Experimental Conditions

a. Buffers

Experiments are performed in appropriate, non-cytotoxic, physiological saline iso-osmotic buffers at pH 7.4 (basolateral/serosal side) or pH 6.5 (apical/mucosal side). Preferred buffers are Ringer's buffer (pH 7.4), Ringer's with glucose (pH 7.4), MES ringer's buffers (pH 6.5), or MES Ringer's with glucose (pH 6.5) (**Table 5**).

Table 5: Formulas for Ringer's buffer and Ringer's with glucose buffer.

Chemical	Ringer's buffer (mM)	Ringer's with glucose (mM)	MES Ringers Buffer (mM)	MES Ringer's With glucose (mM)
KCI	5	5	5	5
Na_2HPO_4	1.15	1.15	--	--
Na_2HPO_4	0.3	0.3	--	--
$NaHCO_3$	25	25	--	--
$MgSO_4$	1.1	1.1	1.1	1.1
$CaSO_4$	1.25	1.25	1.25	1.25
NaCI	qs iso-osmotic	qs iso-osmotic	qs iso-osmotic	qs iso-osmotic
MES	--	--	25	25
Glucose	--	25	--	25

pH adjusted with 1 N HCI or 1 N NaOH

b. Sampling

Samples are collected from the receiver chamber beginning once steady state has been achieved and continuing for at least 90 minutes. Four to six (preferred) samples are collected to allow accurate determination of dC/dt (**Equation 2**). The volume removed from the receiver chamber at each time point is replaced with buffer containing no drug to maintain constant volume in the receiver chamber. The dilution of the receiver concentration due to the addition of buffer is corrected during data analysis and Pe calculation. The concentration may be corrected by: (1) adding the mass removed at each sampling time to the mass removed from the receiver chamber at all prior sampling times, by summing calculated mass absorbed and adding to mass for sample calculation; and (2) using **Equation 4** (preferred).

$$\frac{1}{X} = -\sum_{n}^{k} (-1)n \, \frac{\beta}{n} \, \left(\frac{(S)}{(V)}\right)^{n-1} \qquad \text{(Eq. 4)}$$

where the corrected receiver chamber concentration is obtained by dividing the collected sample concentration by **Equation 4** (1/X), S is the volume of sample withdrawn, V is the receiver chamber volume, k is the sequential sample number, i.e., k=1 for the first sample time, k=2 for the second sample time, k-3 for the third sample time, etc., and β is the corresponding number from Pascal's triangle (**Table 6**).

Table 6: Pascal's Triangle for determining β coefficients.

Sample	1st term	2nd term	3rd term	4th term	5th term	6th term
1	1					
2	1	1				
3	1	2	1			
4	1	3	3	1		
5	1	4	6	4	1	
6	1	5	10	10	5	1

Donor concentration (C_0) is determined by sampling the donor buffer containing the test compound with subsequent analysis directly from the donor chamber, or from a stock solution of donor buffer provided binding and absorption to the interior of the chambers does not occur.

c. Intestinal Tissue

Rabbit intestinal tissue is used for permeability experiments. During mounting of tissue onto chambers, intestinal muscles are stripped off the mucosa and discarded. Care should be taken to ensure integrity of the tissue. A minimum of three chambers are used to determine P_e values for each region, concentration and compound. The mean P_e and Standard Error of the Mean are provided for each study.

d. Cell monolayers

Caco-2 cell monolayer Pe is determined in diffusion chambers similar to NaviCyte Snapwell™ diffusion chambers and follow all procedures described above except the recommended buffers are Ringer's with glucose or MES Ringer's with glucose as listed in **Table 6**.

Caco-2 cells are grown using DMEM media supplemented with 10% FBS, 5% PCN-STEP, and 1% NEAA under 95-100% humidity and 5% CO_2 at 37°C. Cells are grown in flasks and the culture split at 85-95% confluence. Snapwells™ are seeded at 65,000 cell/cm^2 and used in the permeability experiment within 21-28 days post seeding to allow for differentiation.

4. Determination of absorption mechanism

Absorption mechanism for a compound is determined by one of the following methods. Determination of P_e in both the apical-basal (AB) to basal-lateral (BL) and BL to AB directions using **Equation 2**, or determination of P_e in the AB to BL direction at concentrations, (a) close to the analytical limit, and (b) close to the solubility limit.

Similar P_e values in both the AB to BL and BL to AB indicate a passively absorbed compound and no further studies are required. AB to BL P_e greater than BL to AB indicates carrier-mediated absorption and P_e must be determined for 5 additional C_0 in the AB to BL direction. BL to AB P_e greater than AB to BL indicates carrier mediated secretion and P_e determined for 5 additional C_0's in the BL to AB direction.

Similar P_e values at low and high concentrations indicate a passively absorbed compound, and no further studies are required. Low concentration P_e higher than high concentration P_e indicates carrier-mediated absorption and Pe is determined for 5 additional C_0's in the AB to BL direction. High concentration P_e higher than low concentration P_e may indicate carrier-mediated secretion. BL to AB P_e is then determined at the low concentration and the mechanism determined as described above.

B. Solubility determination

Solubility of a compound is determined using an accurate and scientifically sound method similar to the Phase Rule and Phase-solubility analysis as described in Remington's: The Science and Practice of Pharmacy, 19th edition, Chapter 16.

The solubility is determined at pH 1.5 using Simulated Gastric Fluid (USP XXII) minus pepsin. Solubility at pH 6.0, 6.5, 7.0, and 7.5 is determined in Simulated Intestinal Fluid (USB XXII) minus pancreatin. Parameters are for data collection are carefully monitored by ensuring purity of the test compound and accuracy of the Simulated Gastrointestinal fluids. A temperature of 37°C is maintained accurately during the course of the determination. Complete saturation and accurate analysis of saturated solutions are employed.

C. Dissolution determination

The dissolution rates are determined using the equipment, apparatus, and methods described in USP XXII, <711> dissolution. The dissolution rate at pH 1.5 is determined in Simulated Gastric Fluid (USP XXII) minus pancreatin. Concentrations are collected and analyzed for drug compound from the vessel for a sufficient time (6 hours, preferable) to allow the initial slope of the concentration v. time curve to be determined. The slope (dissolution rate) is determined using the initial linear portion of the concentration v. time plot if non-sink conditions exist. Under sink conditions, the entire plot are used to calculate the slope. The slope is reported as the dissolution rate. Explanations of the dissolution rate, sink and non-sink conditions, and equations for calculation are given in Remington's: the Science and Practice of Pharmacy, 19th edition, Chapter 34.

If a formulated dosage form is used for dissolution testing, the dissolution protocols described are used to determine the dissolution rate for drug compound from the formulated dosage form.

Example 5: Standards and Protocols for Evaluating Permeability Data Collection

This example provides detailed protocols for controlling the quality of permeability data collection described in **Examples 3** and **4**. Compounds listed in **Table 7** are used as standards for monitoring permeability data collection and quality. The compounds were chosen to represent each intestinal transport mechanism (passive transcellular, passive paracellular, carrier-mediate influx, or carrier-mediated efflux).

Table 7: Permeability Standards

Transport mechanism	Compounds
Passive Paracellular	mannitol
Passive Transcellular	hydrocortisone
Carrier-mediated Influx	D-glucose
Carrier-mediated Efflux	etoposide

Mannitol, hydrocortisone, D-glucose, and etoposide also were chosen since they are widely used as markers for intestinal transport across rabbit tissue and other systems with well characterized Pe values. These compounds also are available commercially as either 3H-labeled or 14C-labeled.

Permeability data for standards is compared to the values for rabbit listed in **Table 8** (or other standard values) using basic statistical analyses. If the data is significantly different ($p\text{-value} > 0.05$) for any of the standard compounds, data collection is repeated.

Table 8: Transport Characteristics of Permeability Standards*

Compound (donor concentration)	Pe (cm/s)			
	Duodenum	Jejunum	Ileum	Colon
mannitol (1mM)5	1.73×10^{-6}	3.54×10^{-6}	4.02×10^{-6}	5.53×10^{-6}
hydrocortisone (0.01 μM)5	3.00×10^{-7}	1.31×10^{-6}	2.91×10^{-6}	3.85×10^{-6}
D-glucose (10 mM)5	4.55×10^{-6}	1.02×10^{-5}	1.45×10^{-5}	9.28×10^{-6}
etoposide (100 μM)				

*Note: permeability values are representative of ranges. Other values or extended ranges may be used.

A. Experimental Conditions

Protocols, conditions and calculations for permeability evaluation of standards are as described in **Example 4**, with the following modifications.

Permeability experiments are performed using Ringer's buffer at pH 7.4 on both the apical/mucosal and basolateral/serosal sides. Ringer's buffer is as described above excepting that glucose is substituted with mannitol when Pe values for glucose are being measured.

Samples are collected from the receiver chamber beginning 30 minutes after experiment initiation and continuing every 15 minutes until 6 samples have been collected (105 minutes). One-half ml is removed from each receiver chamber at each time point and compound concentration determined. The volume removed from the receiver chamber is replaced with buffer containing no drug to maintain constant volume in the receiver chamber. The dilution of the receiver concentration due to the addition of buffer should be corrected during data analysis and Pe calculation. The concentration is corrected by using **Equation 5**.

$$\frac{1}{X} = \sum_{n=1}^{k} (-1)^{n-1} \frac{\beta}{k+1} \left(\frac{S}{V} \right)^{n-1} \qquad \text{(Eq. 5)}$$

Where the corrected receiver chamber concentration is obtained by dividing the collected sample concentration by **Equation 5** (1/X), S is the volume of sample withdrawn, V is the receiver chamber volume, k is the sequential sample number, i.e. k=1 for the first sample time, k=2 for the second sample time, k=3 for the third sample time, etc., and β is the corresponding number from the modified Pascal's triangle below (**Table 9**). Note: Since the sample intervals are not even (i.e. the 1st interval is 30 minutes, all others 15 minutes) **Equation 5** as well as the β coefficients are modified from those listed in **Example 4**.

Table 9: Modified Pascal's Triangle for determing β coefficients

Sample	1st term	2nd term	3rd term	4th term	5th term	6th term
1	2					
2	3	2				
3	4	5	2			
4	5	9	7	2		
5	6	14	16	9	2	
6	7	20	30	27	11	2

The donor concentration C_0 is determined by sampling 0.02 ml of the donor buffer containing drug (with subsequent analysis) directly from the donor chamber. Potential binding of drugs to the chambers also is monitored. Donor samples (0.02 ml) are taken at experiment initiation and at experiment conclusion. If a significant decrease in drug concentration has occurred (>10%) the experiment is repeated using procedures which compensate for the drug loss in the donor chamber. It is recommended that the donor chamber solution be removed and replaced with fresh donor buffer containing drug at appropriate intervals. The intervals and volumes to be used are determined using sound scientific judgment. Adequate data is collected to show the donor drug concentration has remained constant throughout the experiment.

For tissue-based permeability assays, during mounting of tissue onto chambers, intestinal muscles should be stripped off the mucosa and discarded. Care should be taken to ensure integrity of the tissue.

Animals donating tissue are euthanized immediately prior to experiment initiation. The small intestine is excised from the animal and kept in ice cold Ringer's buffer pH 7.4 until mounted in diffusion chambers. As soon as possible after excision, the tissue is cut into an appropriately sized piece and placed over the diffusion chamber pins with the mucosal side down. The muscle layers are carefully stripped away using forceps. After the tissue is mounted the two half chambers are placed together and the donor and receiver sides filled with the appropriate pre-warmed (37°C) buffer. If NaviCyte chambers are used, the gas lift system is connected with 95% O_2/5% CO_2 flowing at ~5 – 15 ml/min (depending upon chamber volume) into each half chamber to maintain pH and mixing. Sampling begins 30 minutes after connection of the gas lift system.

The mean Pe and Standard Error of the Mean are determined for each study. Permeabilities from at least 6 chambers from 3 different animals are used in calculating the mean and Standard Error of the Mean.

In addition, the Pe of radiolabeled mannitol is determined simultaneously with the standard compound as a marker of intestinal integrity. Mannitol Pe values may be determined by concurrent diffusion using a donor buffer containing mannitol and the standard drug compound, or by continuing the experiment for 60 minutes after the last standard compound sample is collected using donor buffer containing mannitol and fresh receiver buffer containing no compounds.

Special experimental conditions are followed for certain standard compounds. This includes such conditions as a proton gradient, a sodium gradient, presence of glucose, etc. These conditions are listed in **Table 10** and are substituted or added to the general conditions listed above.

Table 10: Experimental Conditions

Standard Compound	Donor Concentration	Special Conditions
mannitol	1 mM	
D-glucose	10 mM	
hydrocortisone	0.01 μM	
etoposide	100 μM	drug dissolved in DMSO, DMSO concentration in buffer < 0.1%

Example 6: Physiologic-Based Mass Simulation Model

A. Design

A multi-compartment physiologic-based mass simulation model (the "mass model") was designed to integrate mass-flow relationships among GI compartments representing the stomach, duodenum, jejunum, ileum, and colon, and thus throughout the GI tract, and to characterize drug movement in units of mass into peripheral compartments. Converters that interrelated transfer rates and associated rate constants (k), which in turn were modified by various factors including pH, solubility profiles, compartment surface area and drug permeability were incorporated to account for drug movement among compartments. A plasma kinetics model also was included for validation purposes and for correlating clinical plasma data to the mass model. Converters also were used for unit conversion.

Gancyclovir was chosen to develop and test the mass model. Gancyclovir exhibits no *in vivo* biotransformation and is poorly absorbed. Thus, the mass model assumes no metabolism or protein binding. Additionally, dissolution rate and delivery system were not used in the mass model as modifying parameters of drug absorption, i.e., drug assumed to be completely dissolved in the stomach and solubilized according to its solubility profile.

Surface area values for each compartment of the mass model represented a "functional surface area," as opposed to an absolute value. A functional surface area was utilized since (1) fluids entering the gastrointestinal compartments do not cover the surfaces of the compartment instantaneously, but rather over a time course; and (2) solubilized drug within the fluid is not ideally presented to all absorptive areas. Functional surface areas for each compartment were calculated by solving **Equation 6** for the area using various data inputs from the literature.

$$P \bullet A \bullet S_p = \partial M/\partial t \qquad \text{(Eq. 6)}$$

Where P is the permeability coefficient, A is the surface area of the membrane, S_p is the solubility of the drug in the relevant segment of the intestine, and $\partial M/\partial t$ is drug flux, where flux $\partial M/\partial t$ is determined from the permeability of the drug in the particular intestinal compartment, the surface area covered by drug solution and the solubility of at the pH of the intestinal compartment.

For example, several studies have been conducted comparing permeability of various compounds (Rubas *et al.*, *Pharmaceutical Research*, Vol. 10, No. 1 (1993)). Mannitol, which has similar physicochemical properties to Gancyclovir, also has similar permeability characteristics and a bioavailability of approximately 10% in humans when it is orally administered. For mannitol, permeability is well characterized. Thus, data obtained from the literature related to permeability in each compartment, pH-dependent solubility and mass concentration relationships was used to solve Equation (6) for area. Thus, it was this area, and not the theoretical total surface area of each compartment, that was used as the functional area of a compartment, which represented a good approximation of *in vivo* surface area relationships for initial model building.

Permeability values were obtained from published *in vitro* cell diffusion experiments and were accounted for by converters that modified luminal and peripheral flow (K12) for each compartment. For solubility, a solubility curve was used based on experimental data available in the literature. pH was then isolated in a separate converter to modify the solubility curve for the particular compartment. In contrast, for validation purposes, an absolute solubility value was used and pH was entered as 1 to isolate that converter from the validation model.

Absorption "transfer" rates among each two compartment sub-system were collected into a separate flow representing total absorption rate, which in turn was collected into a compartment representing the total amount of drug absorbed for each GI tract compartment, namely, stomach, duodenum, jejunum, ileum, and colon. Absorption rates among stomach, duodenum, jejunum, ileum, and colon modules were connected by flows modified by the associated rate constants between each GI segment.

For validation purposes, a plasma kinetics model was integrated with the mass-flow compartments by linking the total absorption rate to a flow representing the absorption rate constant, which in turn fed into the central plasma compartment. A standard two-compartment plasma kinetics model (Ramsay, *European Journal of Pharmaceutics and Biopharmaceutics,* Vol. 37, No. 3 (1991)) was used for this purpose. (See Figures 5 and 6) The plasma kinetics model incorporated first order transfers between the blood compartment and peripheral compartment. Two flows were used and set up as first order systems and thus different rate constants were applied in each direction. Compartment values were represented as mass units. Blood volume was input in a converter, which modified a converter for concentration along with the mass compartment. An elimination rate constant was also obtained form the literature in a first order process. In addition, while most drugs are given in milligram doses, plasma concentrations are reported in microgram or nanogram per milliliter. This is done since compounds are distributed rapidly into a large volume after entering the blood resulting in a concentration of drug in systemic circulation that is quite low with respect to the concentration at the site of administration. Accordingly, an additional converter was added to convert milligram units to nanogram or microgram units expected for concentrations of the test compound based on human bioavailability data. A compartment also was added to collect elimination data.

B. Mass Model Parameters

Parameters and associated values of the mass model include pH, solubility, permeability, and intestinal transit, and are illustrated in **Table 11**.

Table 11: Mass Model Parameters/Values

Parameter	Value
Dose	1000mg
dt	0.125
Run Time	24 hrs
ka assumed (mass transit)	2.8 or 3
Stomach	
Area	50 cm^2
Solubility	31 mg/ml
Permeability	1.1 X 10^{-6} cm/sec
Duodenum	
Area	125 cm^2
Solubility	3.65 mg/ml
Permeability	1.1 X 10^{-6} cm/sec
Jejunum	
Area	182 cm^2
Solubility	3.65 mg/ml
Permeability	2.17 x10^{-6} cm/sec
Ileum	
Area	102 cm^2
Solubility	3.65 mg/ml
Permeability	4.06x 10^{-6} cm/sec
Colon	
Area	138 cm^2
Solubility	3.65 mg/ml
Permeability	3.80 10^{-6} cm/sec
Plasma Kinetics	
k_{12}	0.839
k_{21}	0.670

k_{elim}	0.161
Fluid Volume	76,800 ml

The mass model also was tested by inputting values derived from the literature (Gibaldi *et al.*, *Pharmacokinetics*, pp. 284-288, Marcell Dekker (1975)) into the plasma kinetics model. These values are shown in **Table 12.**

Table 12: Values for Plasma Kinetic Module

Dose	1g
1505a	2.718 h^{-1}
1505b	0.254 h^{-1}
k_{21}	0.3737h^{-1}
k_{12}	0.7509h^{-1}
k_{10}	1.3474h^{-1}
V_p	20.1241

Example 7: Testing and Validation Mass Model

The mass model was tested using parameters shown in **Table 11** with an initial dose of 1000 mg over a time course of 24 hours. AUC, C_{max}, T_{max}, and $T_{1/2}$ were simulated using various doses (New Drug Application for Gancyclovir Sodium, Syntex (USA), (obtained from the FDA under the Freedom of Information Act (FIA)) and compared to human clinical data obtained for Gancyclovir. Bioavailability simulated by the mass model for Gancyclovir was approximately 6%. Compared to human clinical data, obtained for two Phase I clinical studies (designated here as ICM 1505 and 1505b), bioavailability of fasted patients in clinical trials typically ranged from 3-20%. The mass model also was tested using a plasma kinetics validation model illustrated in **Figure 8.**

Figure 16 shows the area under the concentration time curve for a 1000 mg dose of Gancyclovir, Tmax = 1.4 hrs, Cmax = .51 ng/ml., using the mass model, as compared to clinical study data of ICM 1505 and 1505b. The results demonstrate that the mass model underestimated plasma concentration during the post-absorptive period. **Table 13** shows comparison of some values between clinical studies and those predicted by the mass model. The clinical studies also used a 70Kg body weight for normalization of concentrations.

Table 13: Comparison of Mass Model to Clinical data

Parameter	Mass Model	Clinical 1505a	Clinical 1505b
Cmax (mcg/ml)	0.51	0.55	0.59
Tmax (hrs)	1.40	1.43	1.43

Example 8: Physiologic-Based Volume Simulation Model

A. Design

A physiologic-based simulation model for incorporating fluid volume flux and GI transit (the "volume model") was developed for integration with the mass model to account for changes in absorption resulting from fluid absorption/secretion and transit, and thus apparent drug concentration. The volume model was constructed so that fluid enters a compartment and was absorbed by a first order process based on an absorption rate for that fluid. Movement of fluid between compartments was dependent on a zero or first order fluid transit rate.

B. Volume Model Parameters

As a starting point for the volume model, values were obtained from literature that described in general terms absorption and secretion of fluid throughout the body (Change *et al.*, *Gastrointestinal, Hepatobiliary and Nutritional Physiology*, Chapter 5, p. 92, Lippincott-Raven (1996)). Values representing total intake of fluid per day and total secretion of fluid per day were modeled into the system normalized linearly to increments of dt for the model. To permit for changes in dt for the model, the values were entered as pulses. Values used in the volume model are shown in **Table 14**.

Table 14: Volume Model Parameters/Values

Source	ml/24hrs	ml/0.1hrs
Intake/Secretion		
Stomach	6500	27.08
Orally	2000	8.33
Salivary Glands	1500	6.25
Stomach	2500	10.42
Duodenum	2000	8.33
Bile	500	2.08
Pancreas	1500	6.25
Jejunum/Ileum	1000	4.17
Jejunum	641	2.67
Ileum	359	1.50
Colon	0	0
Total	9000	337.57.5
Absorption		
Duodenum	2598	10.82
Jejunum	3783	15.76
Ileum	2120	8.83
Colon	400	1.67
Total	8900	37.09
Note: Values for compartments based on %total intestinal area		

Where data was only available for a series of compartments, values were assigned to each compartment based on the percentage of the total area for that series (e.g. secretions for jejunum and ileum and absorption for parts of the small intestine). The model was set as two flows between the blood (serosal) side of the compartment and the compartment itself. Each flow represented the rate constant for secretion and fluid absorption.

For development purposes, absorption and stomach secretion were assumed to be zero order when using values from **Table 14** for both flows. Also, daily volume for fluid entry into the stomach was entered as a pulse according to the dt values shown in **Table 14**. Thus, total intake and secretions of fluid was modeled as a pulse occurring every 6 minutes throughout a 24 hour period. Initial volume in the stomach also was set up as a pulse of the total oral intake, salivary excretion, and stomach secretion over each dt increment.

Example 9: Testing and Validation of Volume Model

To test movement of fluid between compartments the volume model was modified to approximate zero order fluid transit or emptying and isolated from the mass component of the model. Initial values of 1000 ml and 250 ml were used for testing.

Example 10: Physiologic-Based Mass-Volume Simulation Model

A. Design

A physiologic-based simulation model integrating the mass and volume models (the "mass-volume model") was constructed to integrate complex mass and fluid flow relationships. The integrated mass-volume model also included compartments to characterize drug movement into peripheral compartments. A plasma kinetics model for training/validation purposes also was included. The basic design for the integrated mass-volume model, linked to the plasma kinetics model shown in **Figure 8,** is illustrated in **Figure 11.**

Volume for a compartment was added as a product to obtain the amount of drug solubilized at a time increment volume. Additionally, an "IF ... THEN ... ELSE" control statement was added to prevent the equation from indicating that more drug was solubilized than dosed. Thus, the integrated mass-volume model shows the mass of drug in the stomach connected to the absorption rate constant as well as the volume compartment.

Mass and fluid transit rate constants of 2.8 and 3 for the stomach were calculated from values obtained from the literature for Gancyclovir (Syntex, Clinical Studies ICM 1653 and 1774, FDA NDA available data and Bachrach *et al.*, Functional and Diagnostic Aspects of the Upper Digestive Tract, *Digestive System, Part I, Upper Digestive Tract,* Netter (1989)), and determined for each of the remaining compartments to approximate mass and fluid movement.

B. Mass-Volume Model Parameters

Parameters and associated values and equations were systematically varied or as described above for individual mass and volume models; an example of the equations and parameters employed in the mass-volume model are shown in **Appendix 1.** Dissolution rate and delivery system (controlled release device/formulation) were excluded from in the mass-volume model, and thus the model assumes a test compound is immediately in solution in the stomach.

Example 11: Testing and Validation of Mass-Volume Model

The mass-volume model was tested using the equations and parameters shown in **Appendix 1.** These parameters included the pulsed estimate of fluid absorption and gastrointestinal secretions, and rate constants extracted from the literature. Alternate sets of parameters for fluid absorption and secretions also were tested. For example, simple zero and first order rate constants of 1 or a sequential integer and various doses were evaluated for comparison to human clinical data.

Figure 17 shows the area under the concentration time curve for a 1000 mg dose of Gancyclovir, Tmax = 1.1875 hrs, Cmax = .54 mcg/ml., using the mass-volume model of **Figure 11** with the estimated absorption and secretion rates, relationships, and values of **Appendix 1,** as compared to clinical study data of ICM 1505 and 1505b. The data is now less favorable for Tmax but more favorable for AUC compared to the mass model. These results demonstrate that the mass model underestimated plasma concentration during the post-absorptive period, while the combined mass-volume model appeared to overestimate it.

The mass-volume model was modified to incorporate simple zero and first order absorption and secretion. This model was then run using an initial volume of 250 ml and also 4 administrations of 250 ml water as done during clinical studies. Results were similar to the results shown in **Figure 17,** but with slightly higher absorption.

The mass-volume model also was run using the following combinations of data input: (1) doses of 500 mg, 750 mg, 1000 mg at qid, bid, and tid dosing; (2) initial volumes of 250 ml, 500 ml, 1000 ml; (3) varying absorption and secretion rates based on differing assumptions for daily secretion and fluid intake; (4) varying pH values in the various compartments; and (5) simulation of food intake and fasting conditions. Correlation was very good with some clinical data and less than optimal with others. Correlation with theoretical estimations also varied from very good to poor.

Collectively, the mass-volume model represented an improvement over the individual mass and volume models in that it provided a better approximation of *in vivo* conditions. While the simpler mass-model correlated better with clinical data, the integrated mass-volume model was more sensitive to changes in the various input parameters, physiological conditions and underlying constants, and thus a more rigorous model of the GI tract.

Example 12: Physiologic-Based GI tract Simulation Model

A. Design

The mass-volume model was selectively improved in a stepwise fashion to create an integrated physiologic-based simulation model of the GI tract of a mammal (the "GI model") capable of compound-independent prediction of oral absorption with a high level of accuracy. The model was developed to be flexible. That is, it was designed so that additional physiological factors that influence oral absorption could be identified and incorporated into the model as needed to improve the quality of the prediction for a diverse set of test compounds. Additionally, the GI model was developed to minimize input data requirements.

The basic approach involved generation, testing and integration of a GI transit model (**Figure 20**), a pH-dependent solubility and dissolution model (**Figure 21**), and an absorption model (**Figure 22**), as well as underlying equations and parameters, constants, calculated parameters, and rules by which a given simulation is to proceed. A controlled release device and formulation compartment also was included. A graphical compartment-flow model of the integrated GI model is illustrated in **Figure 24** (without converters, ghost or connectors) and **Figure 25** (with converters, ghost and connectors). Parameter inputs, calculations and outputs are illustrated in **Figures 29-39**. An abbreviation key for the GI model is provided as **Appendix 3**.

The GI model also incorporated additional features to improve the predictive power and versatility of the simulation model. One feature was the development and incorporation of regression analysis derived adjustment parameters based on analysis and processing of human clinical data and *in vitro* data for a diverse set of compounds. The adjustment parameters were utilized as constants in the GI model, and thus modify underlying equations of the model. A second feature was development and incorporation of regional permeability correlation parameters and equations that permitted estimation of values for segments of the model that were missing user provided input values for corresponding parameters. This facilitates prediction of oral drug absorption when permeability values or other parameter for a given compound are provided for a limited number of GI segments, for example, when cell-based input data, such permeability data derived from Caco-2 cells is used to provide permeability input data of colon. Another feature was development and incorporation of parameters and calculations to account for transport mechanism and thus transport-specific variations in compound absorption. Another feature was incorporation of the ability to isolate and evaluate specific regional absorption events related to dissolution and mass transit. Also, the GI model was developed to separate absorption into the portal vein (FDp) from hepatic metabolism, so as to account for individual primary barriers to absorption.

B. GI Model Equations, Rules and Parameters

1. General Equations For GI Model:

Various differential equations and rules utilized for the GI tract model are provided below. For the equations, adjustment parameters are designated by the letter Z.

Transit time:

First order transit process

$$\frac{dA}{dt} = k_{TT}[A] \qquad \text{(Eq. 7)}$$

dA/dt = rate of transit (or absorption), k_{TT} = rate of constant, A = amount (compound or water) in proximal compartment.

Rate constant calculation

$$k_{TT} = \frac{\ln 10}{TT_{ADJ}} \qquad \text{(Eq. 8)}$$

TT_{ADJ} = adjusted transit time

$$TT_{ADJ} = (TT_p \cdot Z_{TT} \cdot User_{TT}) \qquad \text{(Eq. 9)}$$

TT_p = physiological transit time, Z_{TT} = transit time adjustment parameter, $User_{TT}$ = User controlled adjustment to transit time.

K_{TT} is a regionally dependent parameter, i.e. different rate constants are used for each region of the GI tract.

Fluid volume absorption/resorption:

$$\frac{dA}{dt} = k_{VA}[A] \qquad \text{(Eq. 10)}$$

dA/dt = rate of absortpion, k_{VA} = rate constant, A = amount of fluid (water) in the compartment

$$k_{VAZ} = k_{emp} \cdot Z_{VA} \qquad \text{(Eq. 11)}$$

Z_{VA} = volume absorption adjustment parameter, k_{emp} is determined empercially to match human fluid absorption *in vivo*.

Dissolution and Solubility:

Dissolution rate (regionally dependent)

$$\frac{d(A)}{dt} = k_D \cdot Z_D \cdot Mass \cdot (S_{ADJ} - C) \qquad \text{(Eq. 12)}$$

A = Amount dissolved, k_D = User supplied dissolution rate constant, Z_D = Dissolution rate adjustment parameter, S_{ADJ} = solubility, C = concentration

Solubility (regionally dependent)

$$S_{ADJ} = \frac{(s_N - s_{n-1})}{(pH_n - pH_{n-1})}(pH - pH_{n-1}) + S_{n-1} \qquad \text{(Eq. 13)}$$

S_{ADJ} = Solubility, S_n = user supplied solubility $\{S_1...S_5\}$, pH_n = user supplied pH values $\{pH_1...pH_5\}$ corresponding to user supplied solubilities, pH = pH value appropriate to region of the system, such as GI tract. n is selected such that $pH_n > pH$, and $pH_{n-1} < pH$. If any of $pH_1...pH_5$ are equal to pH, the corresponding S_n is used as the solubility.

Concentration (regionally dependent)

$$C = \frac{S_{ADJ}}{V} \qquad \text{(Eq. 14)}$$

C = concentration of soluble drug, V = volume of fluid

Flux/Absorption:

$$J = P_{ADJ} \,.\, SA_{ADJ} \,.\, C \qquad \text{(Eq. 15)}$$

J = flux, P_{ADJ} = Adjusted permeability, SA_{ADJ} = Adjusted surface area available for absorption, C = concentration

$$P_{ADJ} = \left(\frac{2}{1+Z_{EFF}}\right) \cdot P_m \cdot Z_F \cdot 3600 + \frac{Z_{ACT} \cdot P_c \cdot 3600}{1+\frac{C}{K_m}} \qquad \text{(Eq. 16)}$$

Z_{EFF} = Efflux transport adjustment parameter, P_m = passive membrane permeability, Z_F = passive permeability or flux adjustment parameter, Z_{ACT} = active permeability adjustment parameter, P_c = active carrier permeability, C = concentration, K_m = Michaelis-Menten kinetic parameter.

Regional Permeability Correlation

Any regional permeability, P_m, can be calculated using any number of other provided permeabilities.

$$\ln P_a = C + A \cdot \ln\frac{1}{P_b} + B \cdot \ln\left(\frac{1}{P_b}\right)^2 \qquad \text{(Eq. 17)}$$

P_a = permeability calculated using the regional correlation, P_b = permeability provided by the user, and A, B, and C = correlation coefficients fitted to determine correlation.

By way of example, rules utilized for a GI tract model of the PK tool and method of the invention include the following general processes.

2. General Processes For Rule Generation:

1. GI transit. The transit of drug compound and fluid volume are somewhat controlled and the transit of formulations and/or controlled release devices is much more strictly controlled.

2. Controlled Drug Release. The release of drug from the dosage form must be controlled such that drug is released into the correct intestinal region at the appropriate time.

3. Dissolution. A comparison between the concentration and the solubility must be made to determine if additional insoluble compound will dissolve, or if compound already dissolved must precipitate to insoluble drug due to solubility limitations.

4. Absorption. Mathematically, absorption may occur when physiologically it is impossible, e.g. when the volume in the colon becomes low enough that any dissolved drug will be within fluid contained in other solid waste also present in the colon and therefore unavailable for absorption. IF...THEN production rules control these situations.

5. Permeability calculations. To estimate unprovided permeability values from provided permeability values logical evaluations must be made to determine the correct equations necessary to make the correlations.

6. Concentration calculations. The concentration in the intestine cannot exceed the solubility for that particular region. If it does, an incorrect flux will be calculated. IF...THEN production rules are used to ensure the correct concentration is used in the flux calculation.

7. Mathematical anomalies. At certain times during the simulation (especially early and late in the simulation) some compartments, flow regulators, or converters used in other calculations may have a value of 0 which will result in a computational error, e.g. division by 0. Production of rules are used to identify these situations and avoid the errors.

The following table lists the specific processes, conditions, results that control statement rules, e.g., IF...THEN production rules, are used to control. Generally, separate rules used for each region of the GI tract and are combined into one line in the table.

Table 15: Rules for Physiologic-Based GI tract Simulation Model

Process	Condition	Result in True	Result if False	Comments
GI Transit of drug compound or fluid volume	Time < 4 hours	No transit to waste	Transit to waste by first order process	Applies to GI regions using different values for the condition.
GI Transit of formulations or controlled release devices	Time, cumulative physiol. transit time	no transit to next compartment	Immediate transit to next compartment	The rate constant for first order transit is set exceedingly large to provide near instantaneous transit.
Controlled release	Time to reach GI region < Time < Time to exit GI region	Drug is released from dosage form to GI region	No drug release into that GI region	Drug is released according to user provided release profile.
Dissolution	Soluble drug/volume (concentration) < Solubility	Drug moves from insoluble to soluble compartment according to dissolution rate	Drug moves from soluble to insoluble compartment according to precipitation rate	Precipitation rate is set to provide near instantaneous precipitation without causing "overshoot".
Absorption	Volume < 1 x 10^{-6} ml AND Mass < 1 x 10^{-8} mg	No absorption, i.e. concentration = 0	Absorption by flux equation	
Permeability Calculations	Duodenum, Jejunum, and Ileum Permeabilities all provided	Use provided Permeabilities	Estimate unprovided permeabilities from provided permeabilities	1 or 2 permeabilities can be used to calculate unprovided permeabilities
Concentration Calculation	Concentration < Solubility	Concentration used in flux equation	Solubility used in flux equation	
Mathematical anomalies	Volume = 0	Dissolution rate = 0	Dissolution rate calculated by Noyes-Whitney equation	Dissolution given as an example. Similar condications are provided for concentration calculations and other processes.

Exemplary equations, rules, parameters and initial values for the graphical compartment-flow model and various sub-models of the integrated GI model illustrated in **Figures 20-25** and **29-39** are provided in **Appendix 4**, as related to the abbreviation key provided as **Appendix 3**. Various aspects of the physiological, adjustment and regional correlation parameters employed in the GI model and their development are described in further detail below.

1. Physiological Parameters

Physiological parameters of the GI model included physiological ranges reported in the literature (**Table 17**) as well as specific values utilized in the model and compiled for each of five regions of the gastrointestinal tract (stomach, duodenum, jejunum, ileum and colon)(**Table 16**). These included values related to pH, transit time, surface area, and volume parameters.

Table 16: Physiological Parameters Employed In GI Model

	pH[a]	Initial Volumes (ml)	Surface Area (cm^2) [b]	Average Transit time (hr) [c]	Volume Transfer Rates (t_{90}) (hr^{-1})[c]	New Water Absorption Rates* (hr^1) [d]
Stomach	1.5	100	NA	0.5	4.6	0
Duodenum	6.0	0	150	0.225	10.8	0
Jejunum	6.5	0	1000	1.5	1.54	1.75
Ileum	7.0	0	1000	1.5	1.54	1.75
Colon	6.5	0	850	24	0.094	0.1

*Water absorption rate parameters were set so that cumulative water absorption from each region using the GI model were in agreement with values listed in **Table 17**.

Table 17: Physiological Parameters Employed In GI Model

	pH^a	Initial Volumes (ml)	Surface Area (cm^2)^b	Average Transit time (hr)^c	Volume Transfer Rates (t_{90}) (hr^{-1})^c	New Water Absorption Rates (hr^1)^d
Stomach	1.0-2.5	100	NA	0.5-3.0	0.8-4.6	0
Duodenum	4.0-6.4	0	147-168	0.20-0.25	9.2-11.5	0
Jejunum	4.4-6.4	0	913.5-1044	1.0-2.0	1.15-2.3	4.0-4.5
Ileum	6.8-7.4	0	913.5-1044	1.2-1.5	1.54-1.9	2.4-2.7
Colon	5.5-7.0	0	763-872	18-36	0.064-0.13	1.4-1.6

a) Lui et al. *J Pharm Sci* 1986;75(3):271-4; Youngberg et al. *Dig Dis Sci* 1987;32(5):472-80; Charman et al. *J Pharm Sci* 1997;86(3):269-82; Langguth et al. *Biopharm Drug Dispos* 1994;15(9):719-46; Kararli TT. *Biopharm Drug Dispos* 1995;16(5):351-80;

b) Wagner JG. *J Pharm Sci* 1961;50(5):59-87; Ho NF, Park JY, Ni PF, et al. Crouthamel W, Sarapu AC, editors. *Animal Models For Oral Drug Delivery In Man: In Situ And In vivo Approaches.* Washington, D.C. American Pharmaceutical Association, 1983; 2, Advancing quantitative and mechanistic approaches in interfacing gastrointestinal drug absorption studies in animals and humans. p. 27-106;

c) Ho et al. Crouthamel W, Sarapu AC, editors. *Animal Models For Oral Drug Delivery In Man: In Situ And In vivo Approaches.* Washington, D.C. American Pharmaceutical Association, 1983; 2, Advancing quantitative and mechanistic approaches in interfacing gastrointestinal drug absorption studies in animals and humans. p. 27-106; Oberle et al. *Journal of Pharmacokinetics & Biopharmaceutics* 1987;15:529-44; Davis SS. *S T P Pharma* 1986;22:1015-22; Davis et al. *Gut* 1986;27:886-92;

d) Turnberg LA. *Digestion* (1973) 9:357-81.

2. Adjustment Parameters

Differences between *in vitro* and *in vivo* conditions, as well as differences between *in vivo* conditions for one species of mammal and a second hamper accurate prediction of absorption using a simulation approach. For example, *in vitro* dissolution rate may or may not be comparable to dissolution rates existing *in vivo*, or, the permeability in rabbits may or may not be comparable to the permeability in humans.

To compensate for such differences, a set of selectively optimized adjustment parameters were developed. These parameters were designed to be utilized as constants that modify the underlying equations of specific compartments of the GI model to permit automatic correlation of input data to output data as well as facilitate accurate prediction of oral absorption for a diverse set of compounds. For example, the differential equation utilized to calculate fluid volume absorption/resorption employs a rate constant obtained from an equation that is modified by a volume absorption adjustment parameter Z_{VA} (see Eq. 11) Listed below **(Table 18)** are examples of parameters that can be used to adjust parameters and equations as well as those which can be added or removed to a given model if necessary.

Table 18: Adjustment Parameters

Compartment	Segment
Regional fluid absorption	stomach duodenum jejunum ileum colon
Flux/Permeability	duodenum jejunum ileum colon
Active/Carrier mediated Transport (absorption)	duodenum jejunum ileum colon
Compound Efflux (secretion)	duodenum jejunum ileum colon
Transfer rates	stomach to duodenum duodenum to jejunum jejunum to ileum ileum to colon colon to waste
Surface Area	duodenum jejunum ileum colon

The adjustment parameters were developed and optimized using a stepwise selective optimization process. Initial adjustment parameters were developed for correlation between humans and rabbit as follows. Two primary sets of data were used: 1) FDp and best fit plasma profiles from *in vivo* clinical pharmacokinetic (PK) data, and 2) simulated FDp and plasma profiles generated from the GI model. The FDp and best fit plasma profiles from *in vivo* PK data was obtained by analyzing and processing IV and PO data from humans for the test set of compounds described in **Example 2** using a regression-based curve fitting algorithm to determine the best fit curve that matched the actual clinical plasma profiles. The second set of data was generated using a developmental GI model.

In vitro data (permeability, solubility, dissolution rate, and dose) were used as inputs into the GI model with the adjustment parameters set to some initial value previously determined to provide reasonably predictable values for FDp. The GI model was used to provide FDp data for each test compound. The FDp data generated from the GI model also was used as input data into an IV/PO PK model, such as the one shown in **Figure 18**, to determine plasma profiles.

The PO input to the IV/PO PK model of **Figure 18** used for fitting clinical data is an error function and shown in **Equation 18**.

$$F = \frac{D \cdot FDp}{2}\left|1 - erf\frac{\frac{1-\frac{t}{t_{50}}}{2}}{\frac{1}{P_e} \cdot \sqrt{\frac{t}{t_{50}}}}\right|$$

(Eq. 18)

Where D is the dose of drug delivered to the intestine, t is time in minutes, t50 is the time for 50% of the drug to be absorbed, and Pe is a parameter (Peclet number) related to the slope of the linear portion of the absorption curve.

When fitting the data, all available *in vivo* PK data (multiple intravenous (IV) dosing and multiple oral (PO) dosing) was analyzed simultaneously using the IV/PO PK model of **Figure 18**. The data were weighted by 1/Standard Error of the Mean (SEM) or by 1/Concentration^2.

The initial adjustment parameter values were determined empirically. Using a limited set of compounds and corresponding *in vitro* data from rabbit tissue, the adjustment parameters were manually varied to obtain FDp values that were reasonably consistent with the actual PK data. After the initial values were determined, the GI model developed using STELLA® was converted to a program file readable by a program having fitting algorithm, such as KINETICA™. The initial adjustment parameters were then simultaneously fit using non-linear regression analysis in a stepwise manner to determine optimized values (i.e., best fit values) for the adjustment parameters. Within each step, a few parameters were selected for optimization by simultaneous fitting. The fitting was approached using an iterative process, where selected adjustment parameters were varied systematically such that the deviation of the GI model determined absorption from the actual PK determined absorption was minimized. Once the deviation was reduced to a satisfactory level, few more parameters were then selected and optimized. The process was continued until all parameters were successfully optimized. The new parameters were then placed into the GI model and the FDp determined for each compound which is compared to the PK FDp to establish the goodness of fit. This process was repeated until an acceptable goodness of fit was established. Using this approach, adjustment parameters were developed to correlate, for example, *in vitro* solubility, dissolution, dose and permeability in rabbits to *in vivo* human absorption. Although FDp was employed as the reference for deviation, the actual measurement of absorption can be evaluated using any number of parameters, such as plasma levels, absorption constants, or others. Moreover, it will be appreciated that many sets of adjustment parameters may be developed and established. For instance, other sets of adjustment parameters may be established to correlate dog, rat, monkey or other species permeability data to human, dog, rat, rabbit, monkey, or other animal *in vivo* absorption.

3. Regional Permeability Correlation Parameters

Since Pe in all intestinal regions may not be available, for instance when cell monolayer data is employed to determine Pe in colon, a correlation was developed that provides a reasonable prediction of unknown Pe values in the other intestinal regions.

An objective was to establish a correlation between regional permeabilities that allowed prediction of permeability in the duodenum, jejunum or ileum using known permeabilities in one or two of the other regions.

Correlation development involved obtention of regional permeability values in intestinal tissue from the literature and experimentally using methods consistent with the experimental protocols as described in **Examples 4-5**.

The regional correlation parameters were estimated using a polynomial equation developed for this purpose (Equation 17). Any regional permeability, P_m, can be calculated using any number of other provided permeabilities.

The regional correlation parameter function was incorporated into the GI model using a logic function module. A control statement was utilized to regulate activation of the regional correlation parameter estimation function when a user provides less than the total number of permeability values for the segments of the GI tract.

The following (**Table 19**) shows correlations that were established along with the corresponding correlation coefficient. Correlations were accomplished by data transformation and fitting to a non-linear function.

Table 19: Results of Regional Correlation

Variable		Correlation Coefficient
Dependent	Independent	
Duodenum	Jejunum	0.870
Duodenum	Ileum	0.906
Jejunum	Duodenum	0.858
Jejunum	Ileum	0.914
Ileum	Duodenum	0.855
Ileum	Jejunum	0.894

As an example of the capability of the correlation, two of the above correlations were evaluated by estimating the permeability in the duodenum and jejunum using ileum Pe values. The compounds chosen were those for which complete Pe values were available.

The error and % error for the permeability calculations were determined by comparing predicted values to the known permeabilities (**Table 20**).

Table 20: Evaluation of Regional Correlations

Compound	Intestinal Region			
	Duodenum		Jejunum	
	Error	%Error	Error	%Error
Compound α1	-4.64E-07	-46.36	2.42E-07	35.03
Compound α2	6.37E-08	5.79	-1.11E-07	-5.14
Compound α3	3.10E-07	114.91	-8.38E-07	-45.28
Compound α4	1.18E-05	196.00	-5.40E-06	-16.38

The above results demonstrate that the regional correlation parameter function of the GI model was able to accurately predict Pe values for compounds within the initial data set (i.e., high r^2).

Example 13: Validation and Testing of GI Model

To demonstrate that the physiological parameters of the model were operating in a logical manner consistent with expected behavior *in vivo*, the parameters were varied and the effect on output monitored. For example, a decrease in the surface area available for absorption should result in a decrease in the amount of compound absorbed. Thus, the physiological parameters of the model were varied by increasing and/or decreasing their values. The effect of these variations on the rate, as measured by T50 (time for 50% absorption), and extent, as measured by FDp, were simulated. **Table 21** shows the physiological parameters that were varied and the expected effect on FDp and T50.

Table 21: Physiological Parameter Variations*

Parameter	Range evaluated	Expected effect	
Surface Area or Permeability	0.05 to 10 X Normal* $1x10^{-7}$ to $1s10^{-5}$ cm/s	Increase in: Surface Area or Permeability	Increase FDp Decrease T50
GI Transit Time	0.05 to 10 X Normal*	Increase in: GI Transit Time	Increase FDp Increase T50
Dissolution Rate	0.05 to 10 X Normal*	Increase in: Dissolution Rate	Increase FDp Decreased T50
Solubility	1 to 100 mg/ml	Increase in: Solubility	Increase FDp Decrease T50

*Normal values used in the model are listed in **Example 12**. In each case, only the parameter chosen was varied, all other parameters were held constant.

All effects on FDp and T50 were as expected with the changes in the physiological parameters. While not all of the ranges were in the physiological range, the lower part of the range was included to assure that the model would limit to zero FDp as the various parameters approached zero.

The basic structure of the GI model also was assessed by comparing its ability to predict, from dose and *in vitro* solubility and rabbit tissue permeability data, the rate and extent of oral drug absorption in humans and dogs for several drugs, including atenolol, ganciclovir, verapamil, and naproxin. These compounds were chosen for their well known and diverse *in vivo* absorption properties and interspecies absorption variability. By changing the physiological parameter values of the simulation model so that they corresponded to the species under investigation, but not changing the model structure, i.e., compartment, flow regulator, converter relationships, efficacy of the model structure could be evaluated. Initial parameter values for dog and human were derived from the literature. Adjustment parameters were used to build the correlation between the *in vitro* data and *in vivo* absorption. For all four drugs, the GI model accurately predicted FDp for both dog and human.

To assess the basic power of the GI model for predicting oral drug absorption, the model was tested by simulating FDp as a function of time so as to separate absorption across intestinal tissue from first pass metabolism and drug concentration in systemic circulation. Accordingly, methods were developed and used to determine

FDp from clinical plasma data so that transport across the intestinal tissue could be determined. This was accomplished by simultaneously fitting clinical pharmacokinetic data (PO and IV) to the two compartment open IV/PO PK model illustrated as a compartment-flow model in **Figure 18**. Elimination was from the central compartment. Input from oral doses was into a pre-systemic compartment (for metabolism) which was in equilibrium with the central compartment. FDp was determined simultaneously for each oral dose. Clinical pharmacokinetic data fitted to the IV/PO PK model demonstrated the ability of the model to accurately determine blood levels in the central compartment.

The fitted clinical FDp data for a test set of compounds was then compared to FDp predicted by the GI model using both experimental *in vitro* values for permeability as input as well as estimated permeability values calculated by the model using the regional permeability correlation function. The permeability source of the test compounds are shown in **Table 22** below.

Table 22: Permeability Source of Test Compounds

Compound	**Permeability source***
∝1	experimental
∝2	experimental
∝3	experimental
∝4	experimental
∝5	estimation
∝6	experimental
∝10	estimation
β1	estimation
β2	estimation
β3	estimation
β5	estimation
β6	estimation

*Experimental – permeability values for all intestinal segments were submitted. Estimation – permeability values were calculated using regional permeability correlation parameters.

Figures 48-52 are illustrative of the results of these tests. The physiological model was found to accurately predict FDp for the test set of compounds. The accuracy of the prediction is based on both rate and extent of absorption. Correlation of FDp extent between the clinical data and as predicted by the model for the test set of compounds yielded a collective regression coefficient (r^2) of greater than 0.92.

Example 14: Smoothing Functions for GI Model

In the *in vivo* physiological situation, permeability and pH do not change at distinct points or places within the GI tract (with the exception of the gastro-duodenal junction). For example, permeability of a drug in the duodenum may be measured at 1.5×10^{-6} cm/s and 2.5×10^{-6} cm/s in the jejunum, but there is no distinct point in the intestine where such an abrupt change exist. Since the GI model simulates five regions or segments of the GI tract, and each segment utilizes its own set of initial permeability and pH values, an abrupt change, as opposed to an incremental transition, is simulated for a dosage form or dissolved drug as it passes distally through the segmented GI tract.

To account for this phenomenon, and to generate a GI model that is as physiologically accurate as possible, smoothing functions were incorporated into the model. Pairs of exponential functions were used to adjust the permeability and pH values in each segment of the intestine. The functions were developed to be time/position dependent using the mean cumulative transit time as cues for adjustment. For example, prior to the cumulative transit time to reach the ileum ($C_{TT}I$), the ileum permeability will be equal to the user provided or regional correlation estimated jejunum permeability. As time approaches $C_{TT}I$, the ileum permeability will correspond to the exact average of the jejunum and ileum permeability at that point. Immediately after $C_{TT}I$, the ileum permeability continues to gradually decrease/increase exponentially until it reaches the user provided, or estimated, ileum permeability.

Two exponential functions were used in combination to effectively smooth the permeability and pH values. The GI model was adapted to employ Equation 19 as the time approaches a mean cumulative transit time (C_{TT}), and Equation 20 immediately after C_{TT}.

$$P = A - ke^{(\alpha t)} \qquad \text{(Eq. 19)}$$

$$P = B + ke^{-\alpha(t - TT)} \qquad \text{(Eq. 20)}$$

Where A = permeability or pH in the previous intestinal region or segment, B = permeability or pH in the latter region, k is defined in Equation 21, α = a constant used to determine the steepness of the transition between regions and is inversely proportional to the transit time of the region, t = time, and TT = cumulative transit time to exit the previous region.

$$k = 0.5(A - B)/e^{\alpha TT} \qquad \text{(Eq. 21)}$$

These smoothing functions were utilized to adjust permeability and pH at junctions of the stomach/duodenum, duodenum/jejunum, jejunum/ileum, and ileum/colon.

APPENDICIES

Appendix 1: Abbreviation Key for Mass-Volume Model

Abbreviation
Kf sd = associated rate constant for stomach and duodenum
Ka dj = associated rate constant for duodenum and jejunum
Ka ji = associated rate constant for jejunum and ileum
Ka ie = associated rate constant for ileum and colon
Ka co = associated rate constant for colon and excretion
SD trans = transfer rate between stomach and duodenum
DJ trans = transfer rate between duodenum and jejunum
JL trans = transfer rate between jejunum and ileum
IC trans = transfer rate between ileum and colon
Waste = transfer rate between colon and excretion
pH s = pH stomach
pH s2 = pH duodenum
pH s3 = pH jejunum
pH s4 = pH ileum
pH s5 = pH colon
sol profile = solubility profile for stomach
sol profile 2 = solubility profile for duodenum
sol profile 3 = solubility profile for jejunum

sol profile 4 = solubility profile for ileum

sol profile 5 = solubility profile for colon

stom ka = associated rate constant for stomach compartments 1 and 2

duo ka = associated rate constant for duodenum compartments 1 and 2

Jej ka = associated rate constant for jejunum compartments 1 and 2

Il ka = associated rate constant for ileum compartments 1 and 2

Colon ka = associated rate constant for colon compartments 1 and 2

SA stom = surface area of stomach

SA duo = surface area of duodenum

SA jej = surface area of jejunum

SA il = surface area of ileum

SA colon = surface area of colon

Perm stom = permeability of stomach

Perm duo = permeability of duodenum

Perm jej = permeability of jejunum

Perm il = permeability of ileum

Perm colon = permeability of colon

Ka sd = associated rate construct for stomach fluid absorption

Ka du = associated rate construct for duodeunm fluid absorption

Ka je = associated rate construct for jejunm fluid absorption

Ka il = associated rate construct for ileunm fluid absorption

Ka co = associated rate construct for colon fluid absorption
Note: other abbreviations adhere to above descriptors and are self explanatory

Appendix 2: Equations, Parameters and Values For Mass-Volume Model

amt_plasma(t) = amt_plasma(t - dt) + (trans_21 + ka - elimination - trans_12) * dt
INIT amt_plasma = 0

INFLOWS:
trans_21 = k21*comp_2
ka = tot_abs_rate

OUTFLOWS:
elimination = amt_plasma*k_elim
trans_12 = k12*amt_plasma
blood_side_col(t) = blood_side_col(t - dt) + (colon_ka_5) * dt
INIT blood_side_col = 0

INFLOWS:
colon_ka_5 = IF Vol_colon*sol_profile_5 >=Colon THEN Colon*SA_colon*perm_colon*3600 ELSE Vol_colon*sol_profile_5*SA_colon*perm_colon*3600
blood_side_dou(t) = blood_side_dou(t - dt) + (duo_ka) * dt
INIT blood_side_dou = 0

INFLOWS:
duo_ka = IF Vol_duod*sol_profile_2 >= duodenum THEN duodenum*SA_duo*perm_duo*3600 ELSE Vol_duod*sol_profile_2*SA_duo*perm_duo*3600
blood_side_il(t) = blood_side_il(t - dt) + (Il_ka) * dt
INIT blood_side_il = 0

INFLOWS:
Il_ka = IF Vol_ileum*sol_profile_4 >=Ileum THEN Ileum*SA_Il*perm_Il*3600 ELSE Vol_ileum*sol_profile_4*SA_Il*perm_Il*3600
blood_side_jej(t) = blood_side_jej(t - dt) + (Jej_ka) * dt
INIT blood_side_jej = 0

INFLOWS:
Jej_ka = IF Vol_jej*sol_profile_3 >=Jejunum THEN Jejunum*SA_jej*perm_jej *3600 ELSE Vol_jej*sol_profile_3*SA_jej*perm_jej*3600
blood_side_sto(t) = blood_side_sto(t - dt) + (stom_ka) * dt
INIT blood_side_sto = 0

INFLOWS:
stom_ka = IF Vol_stom*sol_profile >= Stomach THEN Stomach*SA_stom*perm_stom*3600 ELSE Vol_stom*sol_profile*SA_stom*perm_stom*3600
Colon(t) = Colon(t - dt) + (IC_trans - Waste - colon_ka_5) * dt
INIT Colon = 0

INFLOWS:

```
IC_trans = ka_ic*Ileum

OUTFLOWS:
Waste = ka_col*Colon
colon_ka_5 = IF Vol_colon*sol_profile_5 >=Colon THEN
Colon*SA_colon*perm_colon*3600 ELSE
Vol_colon*sol_profile_5*SA_colon*perm_colon*3600
comp_2(t) = comp_2(t - dt) + (trans_12 - trans_21) * dt
INIT comp_2 = 0

INFLOWS:
trans_12 = k12*amt_plasma

OUTFLOWS:
trans_21 = k21*comp_2
duodenum(t) = duodenum(t - dt) + (SD_trans - duo_ka - DJ_trans) * dt
INIT duodenum = 0

INFLOWS:
SD_trans = if Stomach >0 then kf_sd*Stomach else 0

OUTFLOWS:
duo_ka = IF Vol_duod*sol_profile_2 >= duodenum THEN
duodenum*SA_duo*perm_duo*3600 ELSE
Vol_duod*sol_profile_2*SA_duo*perm_duo*3600
DJ_trans = ka_dj*duodenum
excretion(t) = excretion(t - dt) + (vol_cw) * dt
INIT excretion = 0

INFLOWS:
vol_cw = Vol_colon*ka_col
excretion_2(t) = excretion_2(t - dt) + (Waste) * dt
INIT excretion_2 = 0

INFLOWS:
Waste = ka_col*Colon
Ileum(t) = Ileum(t - dt) + (JL_trans - IC_trans - Il_ka) * dt
INIT Ileum = 0

INFLOWS:
JL_trans = ka_ji*Jejunum

OUTFLOWS:
IC_trans = ka_ic*Ileum
Il_ka = IF Vol_ileum*sol_profile_4 >=Ileum THEN Ileum*SA_Il*perm_Il*3600
ELSE Vol_ileum*sol_profile_4*SA_Il*perm_Il*3600
Jejunum(t) = Jejunum(t - dt) + (DJ_trans - JL_trans - Jej_ka) * dt
INIT Jejunum = 0

INFLOWS:
```

```
DJ_trans = ka_dj*duodenum

OUTFLOWS:
JL_trans = ka_ji*Jejunum
Jej_ka = IF Vol_jej*sol_profile_3 >=Jejunum THEN Jejunum*SA_jej*perm_jej
*3600 ELSE Vol_jej*sol_profile_3*SA_jej*perm_jej*3600
serosal_col(t) = serosal_col(t - dt) + (Adsorp_col - col_secretion) * dt
INIT serosal_col = 0

INFLOWS:
Adsorp_col = PULSE(1.67,0,.1)+0*Vol_colon*ka_co

OUTFLOWS:
col_secretion = 0
serosal_dou(t) = serosal_dou(t - dt) + (Adsorp_Duo - duo_secretion) * dt
INIT serosal_dou = 0

INFLOWS:
Adsorp_Duo = PULSE(10.82,0,.1)+0*Vol_duod*ka_du

OUTFLOWS:
duo_secretion = PULSE(10.82,0,.1)
serosal_ill(t) = serosal_ill(t - dt) + (Adsorpt_ill - ile_secretion) * dt
INIT serosal_ill = 0

INFLOWS:
Adsorpt_ill = PULSE(8.83,0,.10)+0*Vol_ileum*ka_il

OUTFLOWS:
ile_secretion = PULSE(1.50,0,.1)
serosal_jej(t) = serosal_jej(t - dt) + (Adsorp_jej - jej_secretion) * dt
INIT serosal_jej = 0

INFLOWS:
Adsorp_jej = PULSE(15.76,0,.1)+0*Vol_jej*ka_je

OUTFLOWS:
jej_secretion = PULSE(2.67,0,.1)
serosal_sto(t) = serosal_sto(t - dt) + (Adsorp_Stom - Stom_Secretion) * dt
INIT serosal_sto = 0

INFLOWS:
Adsorp_Stom = 0*Vol_stom*ka_sd

OUTFLOWS:
Stom_Secretion = PULSE(16.67,0,.1)
Stomach(t) = Stomach(t - dt) + (- SD_trans - stom_ka) * dt
INIT Stomach = 1000

OUTFLOWS:
```

```
SD_trans = if Stomach >0 then kf_sd*Stomach else 0
stom_ka = IF Vol_stom*sol_profile >= Stomach THEN
Stomach*SA_stom*perm_stom*3600 ELSE
Vol_stom*sol_profile*SA_stom*perm_stom*3600
total_drug_absorbed(t) = total_drug_absorbed(t - dt) + (tot_abs_rate) * dt
INIT total_drug_absorbed = 0

INFLOWS:
tot_abs_rate = stom_ka+duo_ka+Jej_ka+Il_ka+colon_ka_5
Total_Elimination(t) = Total_Elimination(t - dt) + (elimination) * dt
INIT Total_Elimination = 0

INFLOWS:
elimination =  amt_plasma*k_elim
Vol_colon(t) = Vol_colon(t - dt) + (vol_ij + col_secretion - vol_cw - Adsorp_col) * dt
INIT Vol_colon = 0

INFLOWS:
vol_ij = Vol_ileum*ka_ic
col_secretion = 0

OUTFLOWS:
vol_cw = Vol_colon*ka_col
Adsorp_col = PULSE(1.67,0,.1)+0*Vol_colon*ka_co
Vol_duod(t) = Vol_duod(t - dt) + (vol_sd + duo_secretion - voil_dj - Adsorp_Duo) *
dt
INIT Vol_duod = 0

INFLOWS:
vol_sd = kf_sd*Vol_stom
duo_secretion = PULSE(10.82,0,.1)

OUTFLOWS:
voil_dj = Vol_duod*ka_dj
Adsorp_Duo = PULSE(10.82,0,.1)+0*Vol_duod*ka_du
Vol_ileum(t) = Vol_ileum(t - dt) + (vol_ji + ile_secretion - Adsorpt_ill - vol_ij) * dt
INIT Vol_ileum = 0

INFLOWS:
vol_ji = Vol_jej*ka_ji
ile_secretion = PULSE(1.50,0,.1)

OUTFLOWS:
Adsorpt_ill = PULSE(8.83,0,.10)+0*Vol_ileum*ka_il
vol_ij = Vol_ileum*ka_ic
Vol_jej(t) = Vol_jej(t - dt) + (voil_dj + jej_secretion - vol_ji - Adsorp_jej) * dt
INIT Vol_jej = 0

INFLOWS:
voil_dj = Vol_duod*ka_dj
```

```
jej_secretion = PULSE(2.67,0,.1)

OUTFLOWS:
vol_ji = Vol_jej*ka_ji
Adsorp_jej = PULSE(15.76,0,.1)+0*Vol_jej*ka_je
Vol_stom(t) = Vol_stom(t - dt) + (Stom_Secretion - vol_sd - Adsorp_Stom) * dt
INIT Vol_stom = PULSE(8.33,0,.1)

INFLOWS:
Stom_Secretion = PULSE(16.67,0,.1)

OUTFLOWS:
vol_sd = kf_sd*Vol_stom
Adsorp_Stom = 0*Vol_stom*ka_sd
conc_plasma =  (amt_plasma/volume)*mg_to_ug
k12 = .839
k21 = .67
ka_co = 1
ka_col = 3
ka_dj = 3
ka_du = 1
ka_ic = 3
ka_il = 8.83
ka_je = 1
ka_ji = 3
ka_sd = 1
kf_sd = 2.8
k_elim = .161
mg_to_ug = 1000
perm_colon = 3.80e-6
perm_duo = 1.10e-6
perm_Il = 4.06e-6
perm_jej = 2.17e-6
perm_stom = 1.10e-6
ph_s = 1.5
ph_s_2 = 6.6
ph_s_3 = 6.6
ph_s_4 = 7.5
ph_s_5 = 6.6
SA_colon = 138
SA_duo = 125
SA_Il = 102
SA_jej = 182
SA_stom = 50
volume = 4*19200
sol_profile = GRAPH(ph_s)
(1.00, 63.0), (1.50, 25.0), (2.00, 10.0), (2.50, 5.00), (3.00, 4.00), (3.50, 3.80), (4.00,
3.65), (4.50, 3.50), (5.00, 3.65), (5.50, 3.65), (6.00, 3.65), (6.50, 3.65), (7.00, 3.65),
(7.50, 3.65), (8.00, 3.65), (8.50, 4.00), (9.00, 5.00), (9.50, 12.0), (10.0, 23.5)
sol_profile_2 = GRAPH(ph_s_2)
```

```
(1.00, 63.0), (1.50, 25.0), (2.00, 10.0), (2.50, 5.00), (3.00, 4.00), (3.50, 3.80), (4.00,
3.65), (4.50, 3.50), (5.00, 3.65), (5.50, 3.65), (6.00, 3.65), (6.50, 3.65), (7.00, 3.65),
(7.50, 3.65), (8.00, 3.65), (8.50, 4.00), (9.00, 5.00), (9.50, 12.0), (10.0, 23.5)
sol_profile_3 = GRAPH(ph_s_3)
(1.00, 63.0), (1.50, 25.0), (2.00, 10.0), (2.50, 5.00), (3.00, 4.00), (3.50, 3.80), (4.00,
3.65), (4.50, 3.50), (5.00, 3.65), (5.50, 3.65), (6.00, 3.65), (6.50, 3.65), (7.00, 3.65),
(7.50, 3.65), (8.00, 3.65), (8.50, 4.00), (9.00, 5.00), (9.50, 12.0), (10.0, 23.5)
sol_profile_4 = GRAPH(ph_s_4)
(1.00, 63.0), (1.50, 25.0), (2.00, 10.0), (2.50, 5.00), (3.00, 4.00), (3.50, 3.80), (4.00,
3.65), (4.50, 3.50), (5.00, 3.65), (5.50, 3.65), (6.00, 3.65), (6.50, 3.65), (7.00, 3.65),
(7.50, 3.65), (8.00, 3.65), (8.50, 4.00), (9.00, 5.00), (9.50, 12.0), (10.0, 23.5)
sol_profile_5 = GRAPH(ph_s_5)
(1.00, 63.0), (1.50, 25.0), (2.00, 10.0), (2.50, 5.00), (3.00, 4.00), (3.50, 3.80), (4.00,
3.65), (4.50, 3.50), (5.00, 3.65), (5.50, 3.65), (6.00, 3.65), (6.50, 3.65), (7.00, 3.65),
(7.50, 3.65), (8.00, 3.65), (8.50, 4.00), (9.00, 5.00), (9.50, 12.0), (10.0, 23.5)
```

Appendix 3: Abbreviation Key For GI Model

The legend/key has been divided into sub-sections corresponding to the sub-sections of the model diagram.

Numbered suffixes (1, 2, 3, 4, 5, 6) have been assigned to distinguish between intestinal regions (stomach, duodenum, jejunum, ileum, colon, and waste, respectively).

1 – stomach
2 – duodenum
3 – jejunum
4 – ileum
5 – colon
6 – waste

For example, VOL 1 is the volume in the stomach, MASS 3 is the insoluble mass in the jejunum. In the equations, COMP 1 indicates the stomach, COMP 2 the duodenum, COMP 3, the jejunum, etc.

Ghosts are listed under the sub-section containing the original reservoir, flow regulator, or converter.

Abbreviations listed in italics are regionally dependent and set up as arrays to allow independent values for each intestinal region.

In general, ADJ as a prefix indicates a calculated parameter value (ADJ = adjusted), while ADJ as a suffix indicates an adjustment parameter (ADJ = adjustment).

Intestinal model

Reservoirs/Compartments

VOL ABS	Fluid volume absorbed
VOL	Fluid volume
C REL	Mass of drug contained with a formulation or controlled release device
MASS	Insoluble mass of drug (not contained within the formulation or controlled release device)
SOL	Soluble mass of drug
ABSORPTION	Mass of drug absorbed

Flow regulators

REABS	Rate of water absorption
VOL OUT	Fluid volume transit rate
CR OUT	Formulation or controlled release device transit rate

CR INPUT	Drug release rate from formulation or controlled release device
MASS OUT	Insoluble drug mass transit rate
DISS PRECIP	Dissolution rate
SOL OUT	Soluble drug mass transit rate
FLUX	Absorption rate

ADJ PARMS (Adjustment Parameters)

VOL ADJ	Fluid volume absorption adjustment parameter
DISS ADJ	Dissolution rate adjustment parameter
TRANSIT ADJ	Transit time adjustment parameter
SA ADJ	Surface area adjustment parameter
FLUX ADJ	Passive Absorption adjustment parameter
EFFLUX ADJ	Efflux or secretion adjustment parameter
CARRIER ADJ	Active absorption adjustment parameter

PARMS (Parameters)

VOL PARM	Fluid volume absorption rate constant
SURFACE AREA	Surface area available for absorption
DOSE	The administered dose of drug
INIT VOLUME	The administered volume of water or fluid
TIME IN HOURS	A clock
pH	The physiological pH value
PARACELLULAR	A user controlled switch used to adjust absorption based on absorption mechanism

TRANSIT TIME

TRANSFERS	GI transit rate constant
CUMU TT	Cumulative transit time
ADJ TRANSIT TIME	Adjusted GI transit time incorporating adjustment parameter and user input
USER TT INPUT	User controlled adjustments to the GI transit time

OUTPUT CALCULATIONS

ABSORBED TOTAL	Total mass of drug absorbed (sum of ABSORPTION 1...5)
FDp%	Fraction or the dose absorbed into portal vein x 100
FLUX TOTAL	Total absorption rate (sum of FLUX 1...5)
CUM DISS	Cumulative drug mass dissolved
CR Release	Cumulative drug mass released from formulation
CUM DISS RATE	Sum of DISS PRECIP 1...5

CR cumrate	Summ of CR INPUT 1...5

PERMEABILITY CALCULATION

ADJ PERM	Adjusted permeability incorporating all transport mechanisms and relevant adjustment parameters
ACT PE	Active or carrier-mediated absorptive permeability
Km	Constant from the Michaelis-Menten type permeability equation for active transport
REGIONAL	Passive permeability after regional correlation calculation (same as PASS PE if regional correlation is not used)
PASS PE	Passive permeability entered by user
RC	A logical function used in determining the regional correlation
RCSUM	A logical function used in determining the regional correlation

SOLUBILITY CALCULATION

USER pH	User supplied pH value for which a solubility value is available
USER SOLUB	User supplied solubility value corresponding to the USER pH value
ADJ SOLUB	Solubility calculated (if necessary) at the appropriate pH value using the entered USER pH and USER SOLUB values

CONTROLLED RELEASE CALCULATION

CR RATE	The instantaneous release rate from the formulation
CR DOSE	The total dose contained with the formulation
CR AT TIME	The cumulative drug mass release profile
CR AT LAST	The cumulative drug mass release profile

Note: CR AT TIME holds the value at the current time value (t), CR AT LAST holds the value at the immediately preceeding time value (t-1)

CONC CALCULATION

CONCENTRATIONS	The dissolved drug concentration

DISSOLUTION CALCULATION

PRECIP	Precipitation rate constant

DISSOL	Dissolution rate constant
ADJ DISS PRECIP	Adjusted rate constant incorporating PRECIP, DISSOL and calculated concentration

Appendix 4: Equations, Parameters and Values For GI Model

- ADJ PARMS
  - CARRIER_ADJ[COMPS] = 0
  - DISS_ADJ[COMP_1] = 1
  - DISS_ADJ[COMP_2] = 1
  - DISS_ADJ[COMP_3] = 1
  - DISS_ADJ[COMP_4] = 1
  - DISS_ADJ[COMP_5] = 1
  - EFFLUX_ADJ[COMPS] = 1
  - FLUX_ADJ[COMP_1] = 1
  - FLUX_ADJ[COMP_2] = 10
  - FLUX_ADJ[COMP_3] = 8
  - FLUX_ADJ[COMP_4] = 2
  - FLUX_ADJ[COMP_5] = 1
  - SA_ADJ[COMP_1] = 1
  - SA_ADJ[COMP_2] = 1
  - SA_ADJ[COMP_3] = 1
  - SA_ADJ[COMP_4] = 1
  - SA_ADJ[COMP_5] = 1
  - TRANSIT_ADJ[COMP_1] = 1
  - TRANSIT_ADJ[COMP_2] = 1
  - TRANSIT_ADJ[COMP_3] = 1
  - TRANSIT_ADJ[COMP_4] = 1
  - TRANSIT_ADJ[COMP_5] = 1
  - VOL_ADJ[COMP_1] = 1
  - VOL_ADJ[COMP_2] = 1
  - VOL_ADJ[COMP_3] = 1
  - VOL_ADJ[COMP_4] = 1
  - VOL_ADJ[COMP_5] = 1
- CONC CALCULATION
  - CONCENTRATIONS[COMP_1] = if VOL_1=0.0 then 0 else if ADJ_SOLUB[COMP_1]<SOL_1/VOL_1 then ADJ_SOLUB[COMP_1] else SOL_1/VOL_1 + 0*(SOL_2+SOL_5+SOL_3+SOL_4+VOL_3+VOL_2+VOL_4+VOL_5)
  - CONCENTRATIONS[COMP_2] = if VOL_2 = 0.0 then 0 else if (VOL_2<1e-6 AND SOL_2<1e-7) then 0 else if ADJ_SOLUB[COMP_2]<SOL_2/VOL_2 then ADJ_SOLUB[COMP_2] else SOL_2/VOL_2 +0*(SOL_1+SOL_5+SOL_3+SOL_4+VOL_3+VOL_1+VOL_5+VOL_4)

○ CONCENTRATIONS[COMP_3] = if VOL_3 = 0.0 then 0 else if (VOL_3<1e-6 AND SOL_3<1e-7) then 0 else if ADJ_SOLUB[COMP_3]<SOL_3/VOL_3 then ADJ_SOLUB[COMP_3] else SOL_3/VOL_3 +0*(SOL_1+SOL_2+SOL_4+SOL_5+VOL_5+VOL_4+VOL_1+VOL_2)

○ CONCENTRATIONS[COMP_4] = if VOL_4 = 0.0 then 0 else if (VOL_4<1e-6 AND SOL_4<1e-7) then 0 else if ADJ_SOLUB[COMP_4]<SOL_4/VOL_4 then ADJ_SOLUB[COMP_4] else SOL_4/VOL_4 +0*(SOL_1+SOL_2+SOL_3+SOL_5+VOL_1+VOL_2+VOL_3+VOL_5)

○ CONCENTRATIONS[COMP_5] = if VOL_5 = 0.0 then 0 else if (VOL_5<1e-6 AND SOL_5<1e-7) then 0 else if ADJ_SOLUB[COMP_5]<SOL_5/VOL_5 then ADJ_SOLUB[COMP_5] else SOL_5/VOL_5 +0*(SOL_1+SOL_4+SOL_3+SOL_2+VOL_3+VOL_1+VOL_2+VOL_4)

▣ CONTROL RELEASE CALCULATION

○ CR_DOSE = 0

○ CR_RATE = (CR_AT_TIME-CR_AT_LAST)*20*(CR_DOSE/100)

⊘ CR_AT_LAST = GRAPH(TIME-DT)
(0.00, 0.00), (0.25, 17.7), (0.5, 31.5), (0.75, 42.2), (1.00, 50.6), (1.25, 57.1), (1.50, 62.1), (1.75, 66.1), (2.00, 69.2), (2.25, 71.6), (2.50, 73.4), (2.75, 74.9), (3.00, 76.0), (3.25, 76.9), (3.50, 77.6), (3.75, 78.1), (4.00, 78.5), (4.25, 78.9), (4.50, 79.1), (4.75, 79.3), (5.00, 79.5), (5.25, 79.6), (5.50, 79.7), (5.75, 79.7), (6.00, 79.8), (6.25, 79.8), (6.50, 79.9), (6.75, 79.9), (7.00, 79.9), (7.25, 79.9), (7.50, 80.0), (7.75, 80.0), (8.00, 80.0), (8.25, 80.0), (8.50, 80.0), (8.75, 80.0), (9.00, 80.0), (9.25, 80.0), (9.50, 80.0), (9.75, 80.0), (10.0, 80.0), (10.3, 80.0), (10.5, 80.0), (10.8, 80.0), (11.0, 80.0), (11.3, 80.0), (11.5, 80.0), (11.8, 80.0), (12.0, 80.0), (12.3, 80.0), (12.5, 80.0), (12.8, 80.0), (13.0, 80.0)...

⊘ CR_AT_TIME = GRAPH(TIME)
(0.00, 0.00), (0.25, 17.7), (0.5, 31.5), (0.75, 42.2), (1.00, 50.6), (1.25, 57.1), (1.50, 62.1), (1.75, 66.1), (2.00, 69.2), (2.25, 71.6), (2.50, 73.4), (2.75, 74.9), (3.00, 76.0), (3.25, 76.9), (3.50, 77.6), (3.75, 78.1), (4.00, 78.5), (4.25, 78.9), (4.50, 79.1), (4.75, 79.3), (5.00, 79.5), (5.25, 79.6), (5.50, 79.7), (5.75, 79.7), (6.00, 79.8), (6.25, 79.8), (6.50, 79.9), (6.75, 79.9), (7.00, 79.9), (7.25, 79.9), (7.50, 80.0), (7.75, 80.0), (8.00, 80.0), (8.25, 80.0), (8.50, 80.0), (8.75, 80.0), (9.00, 80.0), (9.25, 80.0), (9.50, 80.0), (9.75, 80.0), (10.0, 80.0), (10.3, 80.0), (10.5, 80.0), (10.8, 80.0), (11.0, 80.0), (11.3, 80.0), (11.5, 80.0), (11.8, 80.0), (12.0, 80.0), (12.3, 80.0), (12.5, 80.0), (12.8, 80.0), (13.0, 80.0)...

▣ DISSOLUTION CALCULATION

○ ADJ_DISS_PRECIP[COMP_1] = if VOL_1=0 then 0 else if (SOL_1/VOL_1<ADJ_SOLUB[COMP_1]) then

```
▣ (DISSOL[COMP_1]*DISS_ADJ[COMP_1]*MASS_1*(ADJ_SOLUB[COMP_1]-
  SOL_1/VOL_1)) else ((SOL_1/VOL_1)-ADJ_SOLUB[COMP_1])*PRECIP
  [COMP_1]+
  0*(MASS_1+MASS_2+MASS_3+MASS_4+MASS_5+SOL_1+SOL_2+SOL_
  3+SOL_4+SOL_5+VOL_1+VOL_2+VOL_3+VOL_4+VOL_5)
○ ADJ_DISS_PRECIP[COMP_2] = if VOL_2=0 then 0 else if
  (SOL_2/VOL_2<ADJ_SOLUB[COMP_2]) then
  (DISSOL[COMP_2]*DISS_ADJ[COMP_2]*MASS_2*(ADJ_SOLUB[COMP_2]-
  SOL_2/VOL_2)) else ((SOL_2/VOL_2)-
  ADJ_SOLUB[COMP_2])*PRECIP[COMP_2]
  +0*(MASS_1+MASS_2+MASS_3+MASS_4+MASS_5+SOL_1+SOL_2+SOL
  _3+SOL_4+SOL_5+VOL_1+VOL_2+VOL_3+VOL_4+VOL_5)
○ ADJ_DISS_PRECIP[COMP_3] = if VOL_3=0 then 0 else if
  (SOL_3/VOL_3<ADJ_SOLUB[COMP_3]) then
  (DISSOL[COMP_3]*DISS_ADJ[COMP_3]*MASS_3*(ADJ_SOLUB[COMP_3]-
  SOL_3/VOL_3)) else ((SOL_3/VOL_3)-
  ADJ_SOLUB[COMP_3])*PRECIP[COMP_3]
  +0*(MASS_1+MASS_2+MASS_3+MASS_4+MASS_5+SOL_1+SOL_2+SOL
  _3+SOL_4+SOL_5+VOL_1+VOL_2+VOL_3+VOL_4+VOL_5)
○ ADJ_DISS_PRECIP[COMP_4] = if VOL_4=0 then 0 else if
  (SOL_4/VOL_4<ADJ_SOLUB[COMP_4]) then
  (DISSOL[COMP_4]*DISS_ADJ[COMP_4]*MASS_4*(ADJ_SOLUB[COMP_4]-
  SOL_4/VOL_4)) else ((SOL_4/VOL_4)-
  ADJ_SOLUB[COMP_4])*PRECIP[COMP_4]
  +0*(MASS_1+MASS_2+MASS_3+MASS_4+MASS_5+SOL_1+SOL_2+SOL
  _3+SOL_4+SOL_5+VOL_1+VOL_2+VOL_3+VOL_4+VOL_5)
○ ADJ_DISS_PRECIP[COMP_5] = if VOL_5=0 then 0 else if
  (SOL_5/VOL_5<ADJ_SOLUB[COMP_5]) then
  (DISSOL[COMP_5]*DISS_ADJ[COMP_5]*MASS_5*(ADJ_SOLUB[COMP_5]-
  SOL_5/VOL_5)) else ((SOL_5/VOL_5)-
  ADJ_SOLUB[COMP_5])*PRECIP[COMP_5]
  +0*(MASS_1+MASS_2+MASS_3+MASS_4+MASS_5+SOL_1+SOL_2+SOL
  _3+SOL_4+SOL_5+VOL_1+VOL_2+VOL_3+VOL_4+VOL_5)
○ DISSOL[COMP_1] = 1
○ DISSOL[COMP_2] = 1
○ DISSOL[COMP_3] = 1
○ DISSOL[COMP_4] = 1
○ DISSOL[COMP_5] = 1
```

```
   ○ PRECIP[COMP_1] = 10
   ○ PRECIP[COMP_2] = 10
   ○ PRECIP[COMP_3] = 10
   ○ PRECIP[COMP_4] = 10
   ○ PRECIP[COMP_5] = 10
▣ INPUTS
▣ INTESTINAL MODEL
  ☐ ABSORPTION_1(t) = ABSORPTION_1(t - dt) + (FLUX_1) * dt
    INIT ABSORPTION_1 = 0
     INFLOWS:
       ⇨ FLUX_1 =
         CONCENTRATIONS[COMP_1]*ADJ_PERM[COMP_1]*SURFACE
         _AREA[COMP_1]
  ☐ ABSORPTION_2(t) = ABSORPTION_2(t - dt) + (FLUX_2) * dt
    INIT ABSORPTION_2 = 0
     INFLOWS:
       ⇨ FLUX_2 =
         CONCENTRATIONS[COMP_2]*ADJ_PERM[COMP_2]*SURFACE
         _AREA[COMP_2]
  ☐ ABSORPTION_3(t) = ABSORPTION_3(t - dt) + (FLUX_3) * dt
    INIT ABSORPTION_3 = 0
     INFLOWS:
       ⇨ FLUX_3 =
         CONCENTRATIONS[COMP_3]*ADJ_PERM[COMP_3]*SURFACE
         _AREA[COMP_3]
  ☐ ABSORPTION_4(t) = ABSORPTION_4(t - dt) + (FLUX_4) * dt
    INIT ABSORPTION_4 = 0
     INFLOWS:
       ⇨ FLUX_4 =
         CONCENTRATIONS[COMP_4]*ADJ_PERM[COMP_4]*SURFACE
         _AREA[COMP_4]
  ☐ ABSORPTION_5(t) = ABSORPTION_5(t - dt) + (FLUX_5) * dt
    INIT ABSORPTION_5 = 0
     INFLOWS:
       ⇨ FLUX_5 = if time<32 then
         CONCENTRATIONS[COMP_5]*ADJ_PERM[COMP_5]*SURFACE
         _AREA[COMP_5]*(32-time)/48*(VOL_5/17.2) else 0
```

```
☐ C_REL_1(t) = C_REL_1(t - dt) + (- CR_OUT_1 - CR_INPUT_1) * dt
  INIT C_REL_1 = CR_DOSE
   OUTFLOWS:
     CR_OUT_1 = IF TIME >=  CUMU_TT[COMP_1] THEN C_REL_1*10000
       ELSE 0
     CR_INPUT_1 = if TIME>CUMU_TT[COMP_1] then 0 else CR_RATE
☐ C_REL_2(t) = C_REL_2(t - dt) + (CR_OUT_1 - CR_OUT_2 - CR_INPUT_2)
  * dt
  INIT C_REL_2 = 0
   INFLOWS:
     CR_OUT_1 = IF TIME >=  CUMU_TT[COMP_1] THEN C_REL_1*10000
       ELSE 0
   OUTFLOWS:
     CR_OUT_2 = IF TIME >=  CUMU_TT[COMP_2] THEN C_REL_2*10000
       ELSE 0
     CR_INPUT_2 = if TIME>CUMU_TT[COMP_2] then 0 else CR_RATE
☐ C_REL_3(t) = C_REL_3(t - dt) + (CR_OUT_2 - CR_OUT_3 - CR_INPUT_3)
  * dt
  INIT C_REL_3 = 0
   INFLOWS:
     CR_OUT_2 = IF TIME >=  CUMU_TT[COMP_2] THEN C_REL_2*10000
       ELSE 0
   OUTFLOWS:
     CR_OUT_3 = IF TIME >=  CUMU_TT[COMP_3] THEN C_REL_3*10000
       ELSE 0
     CR_INPUT_3 = if TIME > CUMU_TT[COMP_3] then 0 else CR_RATE
☐ C_REL_4(t) = C_REL_4(t - dt) + (CR_OUT_3 - CR_OUT_4 - CR_INPUT_4)
  * dt
  INIT C_REL_4 = 0
   INFLOWS:
     CR_OUT_3 = IF TIME >=  CUMU_TT[COMP_3] THEN C_REL_3*10000
       ELSE 0
   OUTFLOWS:
     CR_OUT_4 = IF TIME >=  CUMU_TT[COMP_4] THEN C_REL_4*10000
       ELSE 0
☐    CR_INPUT_4 = if TIME>CUMU_TT[COMP_4] then 0 else CR_RATE
  C_REL_5(t) = C_REL_5(t - dt) + (CR_OUT_4 - CR_OUT_5 - CR_INPUT_5)
  * dt
  INIT C_REL_5 = 0
```

```
    INFLOWS:
      CR_OUT_4 = IF TIME >=  CUMU_TT[COMP_4] THEN C_REL_4*10000
        ELSE 0
    OUTFLOWS:
      CR_OUT_5 = IF TIME >=  CUMU_TT[COMP_5] THEN C_REL_5*10000
        ELSE 0
      CR_INPUT_5 = if TIME>CUMU_TT[COMP_5] then 0 else CR_RATE
☐ C_REL_6(t) = C_REL_6(t - dt) + (CR_OUT_5) * dt
   INIT C_REL_6 = 0
    INFLOWS:
      CR_OUT_5 = IF TIME >=  CUMU_TT[COMP_5] THEN C_REL_5*10000
        ELSE 0
☐ MASS_1(t) = MASS_1(t - dt) + (CR_INPUT_1 - MASS_OUT_1 -
   DISS_PRECIP_1) * dt
   INIT MASS_1 = DOSE
    INFLOWS:
      CR_INPUT_1 = if TIME>CUMU_TT[COMP_1] then 0 else CR_RATE
    OUTFLOWS:
      MASS_OUT_1 = MASS_1*TRANSFERS[COMP_1]
      DISS_PRECIP_1 = ADJ_DISS_PRECIP[COMP_1]
☐ MASS_2(t) = MASS_2(t - dt) + (MASS_OUT_1 + CR_INPUT_2 - MASS_OUT_2
   - DISS_PRECIP_2) * dt
   INIT MASS_2 = 0
    INFLOWS:
      MASS_OUT_1 = MASS_1*TRANSFERS[COMP_1]
      CR_INPUT_2 = if TIME>CUMU_TT[COMP_2] then 0 else CR_RATE
    OUTFLOWS:
      MASS_OUT_2 = MASS_2*TRANSFERS[COMP_2]
      DISS_PRECIP_2 = ADJ_DISS_PRECIP[COMP_2]
☐ MASS_3(t) = MASS_3(t - dt) + (CR_INPUT_3 + MASS_OUT_2 - MASS_OUT_3
   - DISS_PRECIP_3) * dt
   INIT MASS_3 = 0
    INFLOWS:
      CR_INPUT_3 = if TIME > CUMU_TT[COMP_3] then 0 else CR_RATE
      MASS_OUT_2 = MASS_2*TRANSFERS[COMP_2]
    OUTFLOWS:
      MASS_OUT_3 = MASS_3*TRANSFERS[COMP_3]
      DISS_PRECIP_3 = ADJ_DISS_PRECIP[COMP_3]
```

```
☐ MASS_4(t) = MASS_4(t - dt) + (CR_INPUT_4 + MASS_OUT_3 - MASS_OUT_4
  - DISS_PRECIP_4) * dt
  INIT MASS_4 = 0
   INFLOWS:
     ⇨ CR_INPUT_4 = if TIME>CUMU_TT[COMP_4] then 0 else CR_RATE
     ⇨ MASS_OUT_3 = MASS_3*TRANSFERS[COMP_3]
   OUTFLOWS:
     ⇨ MASS_OUT_4 = MASS_4*TRANSFERS[COMP_4]
     ⇨ DISS_PRECIP_4 = ADJ_DISS_PRECIP[COMP_4]
☐ MASS_5(t) = MASS_5(t - dt) + (CR_INPUT_5 + MASS_OUT_4 - MASS_OUT_5
  - DISS_PRECIP_5) * dt
  INIT MASS_5 = 0
   INFLOWS:
     ⇨ CR_INPUT_5 = if TIME>CUMU_TT[COMP_5] then 0 else CR_RATE
     ⇨ MASS_OUT_4 = MASS_4*TRANSFERS[COMP_4]
   OUTFLOWS:
     ⇨ MASS_OUT_5 = if time>4 then MASS_5*TRANSFERS[COMP_5]
       else 0
     ⇨ DISS_PRECIP_5 = ADJ_DISS_PRECIP[COMP_5]
☐ MASS_6(t) = MASS_6(t - dt) + (MASS_OUT_5) * dt
  INIT MASS_6 = 0
   INFLOWS:
     ⇨ MASS_OUT_5 = if time>4 then MASS_5*TRANSFERS[COMP_5]
       else 0
☐ SOL_1(t) = SOL_1(t - dt) + (DISS_PRECIP_1 - SOL_OUT_1 - FLUX_1) * dt
  INIT SOL_1 = 0
   INFLOWS:
     ⇨ DISS_PRECIP_1 = ADJ_DISS_PRECIP[COMP_1]
   OUTFLOWS:
     ⇨ SOL_OUT_1 = SOL_1*TRANSFERS[COMP_1]
     ⇨ FLUX_1 =
       CONCENTRATIONS[COMP_1]*ADJ_PERM[COMP_1]*SURFACE_
       AREA[COMP_1]
☐ SOL_2(t) = SOL_2(t - dt) + (SOL_OUT_1 + DISS_PRECIP_2 - SOL_OUT_2
  - FLUX_2) * dt
  INIT SOL_2 = 0
   INFLOWS:
     ⇨ SOL_OUT_1 = SOL_1*TRANSFERS[COMP_1]
```

```
        DISS_PRECIP_2 = ADJ_DISS_PRECIP[COMP_2]
     OUTFLOWS:
        SOL_OUT_2 = SOL_2*TRANSFERS[COMP_2]
        FLUX_2 =
          CONCENTRATIONS[COMP_2]*ADJ_PERM[COMP_2]*SURFACE_
          AREA[COMP_2]
☐  SOL_3(t) = SOL_3(t - dt) + (DISS_PRECIP_3 + SOL_OUT_2 - SOL_OUT_3
    - FLUX_3) * dt
    INIT SOL_3 = 0
     INFLOWS:
        DISS_PRECIP_3 = ADJ_DISS_PRECIP[COMP_3]
        SOL_OUT_2 = SOL_2*TRANSFERS[COMP_2]
     OUTFLOWS:
        SOL_OUT_3 = SOL_3*TRANSFERS[COMP_3]
        FLUX_3 =
          CONCENTRATIONS[COMP_3]*ADJ_PERM[COMP_3]*SURFACE_
          AREA[COMP_3]
☐  SOL_4(t) = SOL_4(t - dt) + (DISS_PRECIP_4 + SOL_OUT_3 - SOL_OUT_4
    - FLUX_4) * dt
    INIT SOL_4 = 0
     INFLOWS:
        DISS_PRECIP_4 = ADJ_DISS_PRECIP[COMP_4]
        SOL_OUT_3 = SOL_3*TRANSFERS[COMP_3]
     OUTFLOWS:
        SOL_OUT_4 = SOL_4*TRANSFERS[COMP_4]
        FLUX_4 =
          CONCENTRATIONS[COMP_4]*ADJ_PERM[COMP_4]*SURFACE_
          AREA[COMP_4]
☐  SOL_5(t) = SOL_5(t - dt) + (DISS_PRECIP_5 + SOL_OUT_4 - SOL_OUT_5
    - FLUX_5) * dt
    INIT SOL_5 = 0
     INFLOWS:
        DISS_PRECIP_5 = ADJ_DISS_PRECIP[COMP_5]
        SOL_OUT_4 = SOL_4*TRANSFERS[COMP_4]
    OUTFLOWS:
        SOL_OUT_5 = if time>4 then SOL_5*TRANSFERS[COMP_5] else 0
        FLUX_5 = if time<32 then
```

```
          CONCENTRATIONS[COMP_5]*ADJ_PERM[COMP_5]*SURFACE_
          AREA[COMP_5]*(32-time)/48*(VOL_5/17.2) else 0
☐ SOL_6(t) = SOL_6(t - dt) + (SOL_OUT_5) * dt
  INIT SOL_6 = 0
   INFLOWS:
     ⇨ SOL_OUT_5 = if time>4 then SOL_5*TRANSFERS[COMP_5] else 0
☐ VOL_1(t) = VOL_1(t - dt) + (- REABS_1 - VOL_OUT_1) * dt
  INIT VOL_1 = INIT_VOLUME
   OUTFLOWS:
     ⇨ REABS_1 = VOL_1*VOL_PARM[COMP_1]
     ⇨ VOL_OUT_1 = VOL_1*TRANSFERS[COMP_1]
☐ VOL_2(t) = VOL_2(t - dt) + (VOL_OUT_1 - VOL_OUT_2 - REABS_2) * dt
  INIT VOL_2 = 0
   INFLOWS:
     ⇨ VOL_OUT_1 = VOL_1*TRANSFERS[COMP_1]
   OUTFLOWS:
     ⇨ VOL_OUT_2 = VOL_2*TRANSFERS[COMP_2]
     ⇨ REABS_2 = VOL_2*VOL_PARM[COMP_2]
☐ VOL_3(t) = VOL_3(t - dt) + (VOL_OUT_2 - VOL_OUT_3 - REABS_3) * dt
  INIT VOL_3 = 0
  INFLOWS:
     ⇨ VOL_OUT_2 = VOL_2*TRANSFERS[COMP_2]
   OUTFLOWS:
     ⇨ VOL_OUT_3 = VOL_3*TRANSFERS[COMP_3]
     ⇨ REABS_3 = VOL_3*VOL_PARM[COMP_3]
☐ VOL_4(t) = VOL_4(t - dt) + (VOL_OUT_3 - VOL_OUT_4 - REABS_4) * dt
  INIT VOL_4 = 0
   INFLOWS:
     ⇨ VOL_OUT_3 = VOL_3*TRANSFERS[COMP_3]
   OUTFLOWS:
     ⇨ VOL_OUT_4 = VOL_4*TRANSFERS[COMP_4]
     ⇨ REABS_4 = VOL_4*VOL_PARM[COMP_4]
☐ VOL_5(t) = VOL_5(t - dt) + (VOL_OUT_4 - VOL_OUT_5 - REABS_5) * dt
  INIT VOL_5 = 0
   INFLOWS:
     ⇨ VOL_OUT_4 = VOL_4*TRANSFERS[COMP_4]
   OUTFLOWS:
     ⇨ VOL_OUT_5 = VOL_5*TRANSFERS[COMP_5]
```

```
        ⇨ REABS_5 = VOL_5*VOL_PARM[COMP_5]
  ☐ VOL_6(t) = VOL_6(t - dt) + (VOL_OUT_5) * dt
      INIT VOL_6 = 0
      INFLOWS:
        ⇨ VOL_OUT_5 = VOL_5*TRANSFERS[COMP_5]
  ☐ VOL_ABS_1(t) = VOL_ABS_1(t - dt) + (REABS_1) * dt
      INIT VOL_ABS_1 = 0
      INFLOWS:
        ⇨ REABS_1 = VOL_1*VOL_PARM[COMP_1]
  ☐ VOL_ABS_2(t) = VOL_ABS_2(t - dt) + (REABS_2) * dt
      INIT VOL_ABS_2 = 0
      INFLOWS:
        ⇨ REABS_2 = VOL_2*VOL_PARM[COMP_2]
  ☐ VOL_ABS_3(t) = VOL_ABS_3(t - dt) + (REABS_3) * dt
      INIT VOL_ABS_3 = 0
      INFLOWS:
        ⇨ REABS_3 = VOL_3*VOL_PARM[COMP_3]
  ☐ VOL_ABS_4(t) = VOL_ABS_4(t - dt) + (REABS_4) * dt
      INIT VOL_ABS_4 = 0
      INFLOWS:
        ⇨ REABS_4 = VOL_4*VOL_PARM[COMP_4]
  ☐ VOL_ABS_5(t) = VOL_ABS_5(t - dt) + (REABS_5) * dt
      INIT VOL_ABS_5 = 0
      INFLOWS:
        ⇨ REABS_5 = VOL_5*VOL_PARM[COMP_5]
▣ MULTI DOSE CALCULATION
▣ OUTPUT CALCULATIONS
  ☐ CR_Release(t) = CR_Release(t - dt) + (CR_cumrate) * dt
      INIT CR_Release = 0
      INFLOWS:
        ⇨ CR_cumrate = CR_INPUT_1+CR_INPUT_2+CR_INPUT_3+
            CR_INPUT_4+CR_INPUT_5
  ☐ CUM_DISS(t) = CUM_DISS(t - dt) + (CUMM_DISS_RATE) * dt
      INIT CUM_DISS = 0
      INFLOWS:
        ⇨ CUMM_DISS_RATE =
            DISS_PRECIP_1+DISS_PRECIP_2+DISS_PRECIP_3+DISS_PREC
            IP_4+DISS_PRECIP_5
```

○ ABSORBED_TOTAL = ABSORPTION_2+ABSORPTION_3+ABSORPTION_4+ABSORPTION_5
○ FDp% = ABSORBED_TOTAL/DOSE*100
○ FLUX_TOTAL = FLUX_2+FLUX_3+FLUX_4+FLUX_5

▣ PARMS
○ DOSE = 1000
○ INIT_VOLUME = 100
○ PARACELLULAR = 1
○ pH[COMP_1] = 1.5
○ pH[COMP_2] = 5
○ pH[COMP_3] = 6.5
○ pH[COMP_4] = 7
○ pH[COMP_5] = 6.5
○ SURFACE_AREA[COMP_1] = if PARACELLULAR =0 then 50*SA_ADJ[COMP_1] else 50*SA_ADJ[COMP_1]
○ SURFACE_AREA[COMP_2] = if PARACELLULAR=0 then 150*SA_ADJ[COMP_2] else 7.5*SA_ADJ[COMP_2]
○ SURFACE_AREA[COMP_3] = if PARACELLULAR=0 then 1000*SA_ADJ[COMP_3] else 50*SA_ADJ[COMP_3]
○ SURFACE_AREA[COMP_4] = if PARACELLULAR=0 then 1000*SA_ADJ[COMP_4] else 50*SA_ADJ[COMP_4]
○ SURFACE_AREA[COMP_5] = if PARACELLULAR=0 then 850*SA_ADJ[COMP_5] else 42.5*SA_ADJ[COMP_5]
○ TIME_IN_HOURS = TIME
○ VOL_PARM[COMP_1] = 0*VOL_ADJ[COMP_1]
○ VOL_PARM[COMP_2] = 0*VOL_ADJ[COMP_2]
○ VOL_PARM[COMP_3] = 1.75*VOL_ADJ[COMP_3]
○ VOL_PARM[COMP_4] = 1.75*VOL_ADJ[COMP_4]
○ VOL_PARM[COMP_5] = 0.10*VOL_ADJ[COMP_5]

▣ PERMEABILITY CALCULATION
○ ACT_PE[COMPS] = [0,
0,
0,
0,
0]
○ ADJ_PERM[COMP_1] =
(2/(1+EFFLUX_ADJ[COMP_1]))*REGIONAL[COMP_1]*FLUX_ADJ[COMP_1]*3600+(CARRIER_DJ[COMP_1]*ACT_PE[COMP_1]*3600/(1+

```
  (CONCENTRATIONS[COMP_1]/(Km[COMP_1]))))*0
○ ADJ_PERM[COMP_2] =
  (2/(1+EFFLUX_ADJ[COMP_2]))*REGIONAL[COMP_2]*FLUX_ADJ[COMP_
  2]*3600+(CARRIER_DJ[COMP_2]*ACT_PE[COMP_2]*3600/(1+
  (CONCENTRATIONS[COMP_2]/(Km[COMP_2]))))
○ ADJ_PERM[COMP_3] =
  (2/(1+EFFLUX_ADJ[COMP_3]))*REGIONAL[COMP_3]*FLUX_ADJ[COMP_
  3]*3600+(CARRIER_DJ[COMP_3]*ACT_PE[COMP_3]*3600/(1+
  (CONCENTRATIONS[COMP_3]/(Km[COMP_3]))))
○ ADJ_PERM[COMP_4] =
  (2/(1+EFFLUX_ADJ[COMP_4]))*REGIONAL[COMP_4]*FLUX_ADJ[COMP_
  4]*3600+(CARRIER_DJ[COMP_4]*ACT_PE[COMP_4]*3600/(1+
  (CONCENTRATIONS[COMP_4]/(Km[COMP_4]))))
○ ADJ_PERM[COMP_5] =
  (2/(1+EFFLUX_ADJ[COMP_5]))*REGIONAL[COMP_5]*FLUX_ADJ[COMP_
  5]*3600+(CARRIER_DJ[COMP_5]*ACT_PE[COMP_5]*3600/(1+
  (CONCENTRATIONS[COMP_5]/(Km[COMP_5]))))
○ Km[COMPS] = [1,
  1,
  1,
  1,
  1]
○ PASS_PE[COMPS] = [0,
  1.10E-06,
  2.17E-06,
  4.06E-06,
  3.80E-06]
○ RC[COMP_1] = PASS_PE[COMP_1]*0
○ RC[COMP_2] = IF PASS_PE[COMP_2]>0 THEN 1 ELSE 0
○ RC[COMP_3] = IF PASS_PE[COMP_3]>0 THEN 2 ELSE 0
○ RC[COMP_4] = IF PASS_PE[COMP_4]>0 THEN 4 ELSE 0
○ RC[COMP_5] = PASS_PE[COMP_5]*0
○ RCSUM = RC[COMP_2]+RC[COMP_3]+RC[COMP_4]
○ REGIONAL[COMP_1] = PASS_PE[COMP_1]+RCSUM*0
○ REGIONAL[COMP_2] = if RCSUM=2 then
  (EXP( -9.011926 + 2.594378 *LOGN(1/PASS_PE[COMP_2]) -0.065515
  *LOGN(1/PASS_PE[COMP_2])^2))^(-1) else
  if RCSUM=4 then
```

```
      (EXP(-0.369414*LOGN(1/PASS_PE[COMP_4])+0.23756*LOGN(1/PASS_PE
      [COMP_4])^2-0.0009719*LOGN(1/PASS_PE[COMP_4])^3))^(-1) else
      if RCSUM=6 then
      0.5*(EXP( -9.011926 + 2.594378 *LOGN(1/PASS_PE[COMP_3]) -0.065515
      *LOGN(1/PASS_PE[COMP_3])^2))^(-1)
      +0.5*(EXP( -21.009845 + 4.544238 *LOGN(1/PASS_PE[COMP_4]) -0.140815
      *LOGN(1/PASS_PE[COMP_4])^2))^(-1) else
      PASS_PE[COMP_2]
   ○  REGIONAL[COMP_3] = if RCSUM=1 then
      (EXP( -3.238469 + 1.509131 *LOGN(1/PASS_PE[COMP_2]) -0.022109
      *LOGN(1/PASS_PE[COMP_2])^2))^(-1) else
      if RCSUM=4 then
      (EXP(-0.093739*LOGN(1/PASS_PE[COMP_4])+0.182344*LOGN(1/PASS_PE
      [COMP_4])^2-0.0023631*LOGN(1/PASS_PE[COMP_4])^3))^(-1) else
      if RCSUM=5 then
      0.5*(EXP( -3.238469 + 1.509131 *LOGN(1/PASS_PE[COMP_2]) -0.022109
      *LOGN(1/PASS_PE[COMP_2])^2))^(-1)
      +0.5*(EXP( -15.415683 + 3.543563 *LOGN(1/PASS_PE[COMP_4]) -0.100318
      *LOGN(1/PASS_PE[COMP_4])^2))^(-1) else
      PASS_PE[COMP_3]
   ○  REGIONAL[COMP_4] = if RCSUM=1 then
      (EXP( 14.455255 -1.264630 *LOGN(1/PASS_PE[COMP_2]) + 0.082015
      *LOGN(1/PASS_PE[COMP_2])^2))^(-1) else
      if RCSUM=2 then
      (EXP( 11.480333 -0.791109 *LOGN(1/PASS_PE[COMP_3]) + 0.066063
      *LOGN(1/PASS_PE[COMP_3])^2))^(-1) else
      if RCSUM=3 then
      0.5*(EXP( 14.455255 -1.264630 *LOGN(1/PASS_PE[COMP_2]) + 0.082015
      *LOGN(1/PASS_PE[COMP_2])^2))^(-1)
      +0.5*(EXP( 11.480333 -0.791109 *LOGN(1/PASS_PE[COMP_3]) + 0.066063
      *LOGN(1/PASS_PE[COMP_3])^2))^(-1) else
      PASS_PE[COMP_4]
   ○  REGIONAL[COMP_5] = PASS_PE[COMP_5] +RCSUM*0
▣ SOLUBILIY CALCULATION
   ○  ADJ_SOLUB[COMP_1] = if USER_pH[COMP_1]>=pH[COMP_1] then
      USER_SOLUB[COMP_1] else
      ((USER_SOLUB[COMP_2]-USER_SOLUB[COMP_1])/(USER_pH[COMP_2]-
      USER_pH[COMP_1]))*(pH[COMP_1]-USER_pH[COMP_1])+USER_SOLUB
      [COMP_1]
```

○ ADJ_SOLUB[COMP_2] = if USER_pH[COMP_2]=pH[COMP_2] then USER_SOLUB[COMP_2] else if USER_pH[COMP_2]<pH[COMP_2] then ((USER_SOLUB[COMP_3]-USER_SOLUB[COMP_2])/(USER_pH[COMP_3]-USER_pH[COMP_2]))*(pH[COMP_2]-USER_pH[COMP_2])+USER_SOLUB[COMP_2] else ((USER_SOLUB[COMP_2]-USER_SOLUB[COMP_1])/(USER_pH[COMP_2]-USER_pH[COMP_1]))*(pH[COMP_2]-USER_pH[COMP_1])+USER_SOLUB[COMP_1]

○ ADJ_SOLUB[COMP_3] = if USER_pH[COMP_3]=pH[COMP_3] then USER_SOLUB[COMP_3] else if USER_pH[COMP_3]<pH[COMP_3] then ((USER_SOLUB[COMP_4]-USER_SOLUB[COMP_3])/(USER_pH[COMP_4]-USER_pH[COMP_3]))*(pH[COMP_3]-USER_pH[COMP_3])+USER_SOLUB[COMP_3] else ((USER_SOLUB[COMP_3]-USER_SOLUB[COMP_2])/(USER_pH[COMP_3]-USER_pH[COMP_2]))*(pH[COMP_3]-USER_pH[COMP_2])+USER_SOLUB[COMP_2]

○ ADJ_SOLUB[COMP_4] = if USER_pH[COMP_4]=pH[COMP_4] then USER_SOLUB[COMP_4] else if USER_pH[COMP_4]<pH[COMP_4] then ((USER_SOLUB[COMP_5]-USER_SOLUB[COMP_4])/(USER_pH[COMP_5]-USER_pH[COMP_4]))*(pH[COMP_4]-USER_pH[COMP_4])+USER_SOLUB[COMP_4] else ((USER_SOLUB[COMP_4]-USER_SOLUB[COMP_3])/(USER_pH[COMP_4]-USER_pH[COMP_3]))*(pH[COMP_4]-USER_pH[COMP_3])+USER_SOLUB[COMP_3]

○ ADJ_SOLUB[COMP_5] = if USER_pH[COMP_3]=pH[COMP_3] then USER_SOLUB[COMP_3] else if USER_pH[COMP_3]<pH[COMP_3] then ((USER_SOLUB[COMP_4]-USER_SOLUB[COMP_3])/(USER_pH[COMP_4]-USER_pH[COMP_3]))*(pH[COMP_3]-USER_pH[COMP_3])+USER_SOLUB[COMP_3] else ((USER_SOLUB[COMP_3]-USER_SOLUB[COMP_2])/(USER_pH[COMP_3]-USER_pH[COMP_2]))*(pH[COMP_3]-USER_pH[COMP_2])+USER_SOLUB[COMP_2]

○ USER_pH[COMPS] = [1.5,
5,
6.5,
7,
7.5]

- ○ USER_SOLUB[COMPS] = [31,
  3.65,
  3.65,
  3.65,
  3.65]
- ▣ TRANSIT TIME
  - ○ ADJ_TRANSIT_TIME[COMP_1] = .5*TRANSIT_ADJ[COMP_1]*USER_TT_INPUT
  - ○ ADJ_TRANSIT_TIME[COMP_2] = .25*TRANSIT_ADJ[COMP_2]*USER_TT_INPUT
  - ○ ADJ_TRANSIT_TIME[COMP_3] = 1.5*TRANSIT_ADJ[COMP_3]*USER_TT_INPUT
  - ○ ADJ_TRANSIT_TIME[COMP_4] = 1.5*TRANSIT_ADJ[COMP_4]*USER_TT_INPUT
  - ○ ADJ_TRANSIT_TIME[COMP_5] = 24*TRANSIT_ADJ[COMP_5]*USER_TT_INPUT
  - ○ CUMU_TT[COMP_1] = ADJ_TRANSIT_TIME[COMP_1]
  - ○ CUMU_TT[COMP_2] = ADJ_TRANSIT_TIME[COMP_1]+ADJ_TRANSIT_TIME[COMP_2]
  - ○ CUMU_TT[COMP_3] = ADJ_TRANSIT_TIME[COMP_1]+ADJ_TRANSIT_TIME[COMP_2]+ADJ_TRANSIT_TIME[COMP_3]
  - ○ CUMU_TT[COMP_4] = ADJ_TRANSIT_TIME[COMP_1]+ADJ_TRANSIT_TIME[COMP_2]+ADJ_TRANSIT_TIME[COMP_3]+ADJ_TRANSIT_TIME[COMP_4]
  - ○ CUMU_TT[COMP_5] = ADJ_TRANSIT_TIME[COMP_1]+ADJ_TRANSIT_TIME[COMP_2]+ADJ_TRANSIT_TIME[COMP_3]+ADJ_TRANSIT_TIME[COMP_4]+ADJ_TRANSIT_TIME[COMP_5]
  - ○ TRANSFERS[COMP_1] = LOGN(10)/ADJ_TRANSIT_TIME[COMP_1]
  - ○ TRANSFERS[COMP_2] = LOGN(10)/ADJ_TRANSIT_TIME[COMP_2]
  - ○ TRANSFERS[COMP_3] = LOGN(10)/ADJ_TRANSIT_TIME[COMP_3]
  - ○ TRANSFERS[COMP_4] = LOGN(10)/ADJ_TRANSIT_TIME[COMP_4]
  - ○ TRANSFERS[COMP_5] = LOGN(10)/ADJ_TRANSIT_TIME[COMP_5]
  - ○ USER_TT_INPUT = 1

All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

The invention now being fully described, it will be apparent to one of ordinary skill in the art that many changes and modifications can be made thereto without departing from the spirit or scope of the appended claims.

CLAIMS

What is claimed is:

1. A computer-implemented method of predicting a pharmacokinetic property of a target compound in an anatomical segment of a target mammalian system from a pharmacokinetic property of a test compound in an anatomical segment of a data source system, said computer comprising as operably linked components:

(a) an input/output system,

(b) a simulation engine, and

(c) a stored physiologic pharmacokinetic simulation model of said mammalian system, said simulation model comprising:

differential equations for calculating a change in one or more physiological parameters of said target mammalian system and the movement and disposition of said target compound in said mammalian system as a function of time, using input data for said differential equations comprising a pharmacokinetic property of the test compound in the anatomical segment of said data source system; and

a logic function module having control statement rules for initiating said physiologic pharmacokinetic simulation model of said mammalian system function,

wherein said model generates estimated values for a selected pharmacokinetic property of said target compound when supplied with input values corresponding to said selected pharmacokinetic property of said test compound in a portion of said data source system

said method comprising:

(a) entering into said input/output system input data comprising the pharmacokinetic property of said test compound in the segment of said data source system; and

(b) applying said simulation engine and said simulation model, and initiating said estimation function to predict said pharmacokinetic property of said target compound in a segment of said target mammalian system.

2. A computer-implemented method of predicting a pharmacokinetic property of a compound in a first anatomical segment of a mammalian system of interest from a pharmacokinetic property of said compound in a second anatomical segment of said mammalian system of interest, said method comprising:

providing a computer having as operably linked computer-implemented components an input/output system, a simulation engine, and a physiologic pharmacokinetic simulation model of at least first and second anatomical segments of said mammalian system of interest, said simulation model comprising (i) differential equations for calculating the change in one or more physiological parameters of said first and second segments and the movement and disposition of said compound in said first and second segments as a function of time, and (ii) a logic function module having a regional correlation parameter estimation function and a control statement for initiating said function, wherein said estimation function when initiated is capable of generating an estimated value for a selected pharmacokinetic property comprising an absorption parameter of said compound in said first segment when supplied with an input value corresponding to said selected pharmacokinetic property of said compound in said second segment and with a regional correlation coefficient for said selected pharmacokinetic parameter of said first and second segments;

entering into said input/output system input data comprising a pharmacokinetic property of said compound in said second segment; and

applying said simulation engine and said simulation model, and initiating said estimation function to predict said pharmacokinetic property of said compound in said first segment of said mammalian system of interest.

3. The method of claim 2, wherein said regional correlation estimation function comprises a function/transformation algorithm.

4. The method of claim 3, wherein said function/transformation algorithm is selected from the group consisting of a polynomial, exponential, and logarithm.

5. The method of claim 2, wherein said regional correlation coefficient comprises a best fit value that transforms said input data comprising said pharmacokinetic property of said compound in said second segment to an estimated pharmacokinetic property of said compound in said first segment.

6. The method of claim 2, wherein said pharmacokinetic property is selected from the group consisting of absorption, distribution, metabolism, elimination and toxicity.

7. The method of claim 2, wherein said pharmacokinetic parameter is selected from the group consisting of permeability, solubility, dissolution rate and transport mechanism.

8. The method of claim 2, wherein said differential equations are selected from the group consisting of equations for fluid transit, fluid absorption, mass transit, mass dissolution, mass solubility, and mass absorption.

9. The method of claim 2, which further comprises reversibly storing said estimated value for said pharmacokinetic parameter of said compound in said first segment in a computer-implemented database.

10. The method of claim 2, which further comprises reversibly storing in a computer-implemented database an output value corresponding to said pharmacokinetic property of said compound in a segment of said mammalian system that is generated by applying said simulation engine and said simulation model.

11. The method of claim 2, wherein said mammalian system of interest is selected from the group consisting of gastrointestinal tract, liver, heart, kidney, eye, nose, lung, skin and brain.

12. The method of claim 2, wherein said mammalian system of interest is human.

13. The method of claim 2, wherein said input data comprises in vitro data.

14. The method of claim 13, wherein said in vitro data is derived from testing of said compound in an assay that generates data selected from the group consisting of

cell, tissue, physicochemical, structure-activity relationship (SAR) SAR, and quantitative structure-activity relationship (QSAR) QSAR data.

15. The method of claim 2, wherein said computer is a computer system having a data processor, a memory and a display.

16. The method of claim 2, wherein said computer is a standalone computer having a data processor, a memory and a display.

17. The method of claim 2, wherein said computer-implemented components comprise computer readable program code.

18. The method of claim 17, wherein said computer readable program code is embodied in a computer readable medium.

19. A computer-implemented method of simulating one or more parameters of absorption of a compound in a mammalian system of interest using regional correlation parameter estimation, said method comprising:

providing a computer having as operably linked computer-implemented components an input/output system, a simulation engine, and a physiologic pharmacokinetic simulation model of at least two segments of mammalian system of interest having one or more absorption barriers to a compound based on the selected route of administration, said simulation model comprising (i) differential equations for calculating one or more parameters of absorption of said compound in said segments as a function of time and (ii) a logic function module having a regional correlation parameter estimation function and a control statement for initiating said estimation function, said estimation function when initiated being capable of generating an estimated value for a parameter of absorption of said compound in a first segment of said mammalian system utilizing an input value for said parameter of absorption of said compound in a second segment of said mammalian system;

entering through said input/output system input data comprising a parameter of absorption for said compound in said second segment; and applying said simulation engine and said simulation model, and initiating said estimation function to simulate one or more parameters of absorption of said compound in said first segment of said mammalian system of interest.

20. A method of simulating a pharmacokinetic parameter of a compound in a mammalian system of interest, said method comprising:

providing a computer having as operably linked computer-implemented components an input/output system, a simulation engine, and a physiologic pharmacokinetic simulation model of one or more segments of a selected mammalian system having one or more physiological barriers to absorption of said compound based on a selected route of administration, said simulation model comprising: (i) differential equations for one or more of fluid transit, fluid absorption, mass transit, mass dissolution, mass solubility, and mass absorption for one or more segments of said mammalian system; (ii) a regional correlation parameter estimation function for one or more of permeability, solubility, dissolution rate and transport mechanism; (iii) initial parameter values for said differential equations corresponding to physiological parameters and one or more regional correlation parameters for one or more segments of said mammalian system; and (iv) control statement rules for one or more of transit, absorption, permeability, solubility, dissolution, and concentration for one or more segments of said mammalian system;

entering through said input/output system input data comprising dose, permeability and solubility data for said compound for one or more segments of said mammalian system; and

applying said simulation engine and said simulation model to simulate one or more pharmacokinetic parameters of said compound relative to one or more segments of said mammalian system.

21. The method of claim 20, wherein said pharmacokinetic parameters of said compound relative to one or more segments of said mammalian system are selected from the group consisting of absorption, distribution, metabolism, elimination and toxicity.

22. A method of simulating absorption of a compound in a mammal utilizing a pharmacokinetic simulation tool (PK tool), said method comprising:

providing a computer-implemented PK tool comprising an input/output system, a simulation engine, and a simulation model of one or more segments of a mammalian system of interest having one or more physiological barriers to absorption based on the selected route of administration, said simulation model comprising as operably linked components: (i) differential equations for one or more of fluid transit, fluid absorption, mass transit, mass dissolution, mass solubility, and mass absorption for one or more segments of said mammal system; (ii) initial parameter values for said differential equations corresponding to physiological parameters and selectively optimized adjustment parameters, and optionally regional correlation parameters, for one or more segments of said mammal system; and (iii) control statement rules for one or more of transit, absorption, permeability, solubility, dissolution, and concentration for one or more segments of said mammal system;

entering into said input/output system input data comprising dose, permeability and solubility data for said compound for one or more of said segments of said mammal system; and

applying said simulation engine and said simulation model to simulate absorption of said compound in said mammal system.

23. A computer-implemented method of predicting a pharmacokinetic property of a compound in a mammalian system of interest, said method comprising:

providing a computer comprising as operably linked computer-implemented components an input/output system, a simulation engine, and a physiologic pharmacokinetic simulation model of two or more segments of a mammalian system of interest, wherein said simulation model comprises differential equations for calculating as a function of time the change in (i) a physiological parameter of one or more of said segments and (ii) a pharmacokinetic property comprising an absorption parameter of a compound relative to a selected route of administration, barrier to absorption and sampling site of one or more of said segments, and wherein one or more of said differential equations is modified by a selectively optimized adjustment parameter;

entering through said input/output system input data comprising dose, permeability and solubility data for said compound for one or more of said segments of said mammal system; and

applying said simulation engine and said simulation model to predict a pharmacokinetic property of said compound in one or more segments of said mammal system of interest.

24. The method of claim 23, wherein said computer is a computer system having a data processor, a memory and a display.

25. The method of claim 23 wherein said computer is a standalone computer having a data processor, a memory and a display.

26. The method of claim 23, wherein said computer-implemented components comprise computer readable program code.

27. The method of claim 26, wherein said computer readable program code is embodied in a computer readable medium.

28. The method of claim 26, wherein said computer readable program code is embodied in said memory.

29. The method of claim 23, wherein said input/output system comprises a user interface.

30. The method of claim 23, wherein said simulation engine comprises a differential equation solver.

31. The method of claim 23, wherein said differential equations are for fluid transit, fluid absorption, mass transit, mass dissolution, mass solubility, and mass absorption.

32. The method of claim 23, wherein said pharmacokinetic property is selected from the group consisting of absorption, distribution, metabolism, elimination and toxicity.

33. The method of claim 23, wherein said absorption parameter is selected from the group consisting of concentration, permeability, solubility, dissolution rate, transport mechanism, and formulation release rate.

34. The method of claim 23, wherein said physiological parameter is selected from the group consisting of pH, initial fluid volume, surface area, transit time, fluid volume transfer rate, and fluid absorption.

35. The method of claim 23, wherein said mammalian system of interest is human.

36. The method of claim 23, wherein said mammalian system of interest is selected from the group consisting of gastrointestinal tract, liver, heart, kidney, eye, nose, lung, skin and brain.

37. The method of claim 23, wherein said simulation model comprises one or more control statement rules.

38. The method of claim 37, wherein said control statement rules are for controlling simulation of one or more of transit, absorption, permeability, solubility, dissolution, and concentration for one or more segments of said mammal system of interest.

39. The method of claim 23, wherein said input data further comprises data selected from the group consisting of dissolution rate, transport mechanism and formulation release rate.

40. The method of claim 23, wherein said equations comprise one or more input variables corresponding to said input data for calculating as one or more output variables said change in said physiological parameter.

41. The method of claim 23, wherein said equations comprise one or more input variables corresponding to said input data for calculating as one or more output variables said change in said absorption parameter.

42. The method of claim 23, wherein said selectively optimized adjustment parameter correlates said input data to output data comprising said pharmacokinetic property of said compound.

43. The method of claim 42, wherein said input data comprises in vitro data and said selectively optimized adjustment parameter comprises a best fit value obtained by (i) assigning an initial value to a selected adjustment parameter of said simulation model, (ii) fitting a combination of in vitro data and in vivo data for different compounds of a compound test set with said simulation model, (iii) selecting a best fit value for selected adjustment parameter that, when assigned as an initial value to said selected adjustment parameter, permits said simulation model to predict said pharmacokinetic property of said compound when said input data comprises said in vitro data, and (iv) assigning said best fit value to said selected adjustment parameter so as to generate said selectively optimized adjustment parameter.

44. The method of claim 43, wherein said in vitro data is obtained from testing of said compound in one or more assays that generate data selected from the group consisting of cell, tissue, structure-activity relationship (SAR), and quantitative structure-activity relationship (QSAR) data.

45. The method of claim 43, wherein said different compounds of a compound test set comprise compounds having diverse pharmacokinetic properties.

46. The method of claim 42, wherein said input data comprises in vivo data from a first species of mammal and said mammalian system of interest corresponds to a second species of mammal, and wherein said selectively optimized adjustment parameter comprises a best fit value obtained by (i) assigning an initial value to a selected adjustment parameter of said simulation model, (ii) fitting a combination of in vivo data with said simulation model, said combination of in vivo data being derived from testing of different compounds of a compound test set in said first species of mammal and said second species of mammal, (iii) selecting a best fit value for selected adjustment parameter that, when assigned as an initial value to said selected adjustment parameter, permits said simulation model to predict said pharmacokinetic property of said compound when said input data comprises said in vitro data from said first species of mammal, and (iv) assigning said best fit value to said selected adjustment parameter so as to generate said selectively optimized adjustment parameter.

47. The method of claim 46, wherein said different compounds of a compound test set comprise compounds having diverse pharmacokinetic properties.

48. The method of claim 23, wherein said selectively optimized adjustment parameter is for one or more of fluid absorption, flux, permeability, transport mechanism, transfer rate, and segment surface area.

49. The method of claim 22 or 23, which further comprise reversibly storing in a computer-implemented database data corresponding to a predicted pharmacokinetic property of said compound.

50. The method of claim 23, wherein said physiologic pharmacokinetic simulation module comprises at least two of said anatomical segments and a logic function model comprising a regional correlation estimation function and a control statement for initiating said function, wherein said estimation function when initiated is capable of generating an estimated value for a selected pharmacokinetic property of said compound in a first anatomical segment when supplied with an input value corresponding to said selected pharmacokinetic property in a second anatomical segment and with a regional correlation coefficient for said selected pharmacokinetic property of said first and second anatomical segments.

51. The method of claim 50, wherein said regional correlation estimation function comprises a function/transformation algorithm.

52. The method of claim 51, wherein said function/transformation algorithm is selected from the group consisting of a polynomial, exponential, and logarithm.

53. The method of claim 50, wherein said regional correlation coefficient comprises a best fit value that transforms said input data comprising said pharmacokinetic property of said compound in said second segment to an estimated pharmacokinetic property of said compound in said first segment.

54. A method of selectively optimizing a simulation model for predicting pharmacokinetic property of a compound in a mammalian system of interest, said method comprising the steps of:

(i) generating selectively optimized adjustment parameter values for two or more independent parameters of said simulation model utilizing a curve-fitting algorithm that simultaneously estimates the change required in said parameter values necessary for predicting a pharmacokinetic property of said compound in said mammalian system of interest when said simulation model is supplied with one or more input variables corresponding to a pharmacokinetic property of a compound test set derived from (a) a first data source corresponding to the mammalian system of interest, and (b) a second data source corresponding to a system other than the mammalian system of interest;

(ii) selecting adjustment parameter values that permit correlation of one or more of the input variables from the first data source to one or more input variables from the second data source;

(iii) repeating steps (i) and (ii) one or more times for one or more additional independent parameters of said simulation model until deviation of predictability using said first data source as input data into said simulation model from predictability using said second data source as input into said simulation model is minimized; and

(iv) utilizing said selectively optimized adjustment parameters as constants for said independent parameters in said simulation model.

55. A computer-implemented method of generating a selectively optimized value for an adjustment parameter of a physiologic-based simulation model for predicting an in vivo property of a compound in a mammalian system of interest from an in vitro property of said compound, said method comprising:

(i) providing a computer having as operably linked computer-implemented components a curve-fitting algorithm and a physiologic-based simulation model of a mammalian system of interest, wherein said simulation model comprises one or more equations having an input variable for calculating as an output variable an in vivo property of a compound in said mammalian system as a function of time, and wherein one or more of said equations is modified by an adjustment parameter;

(ii) using said computer and said curve-fitting algorithm, fitting with said simulation model a combination of in vitro data and in vivo data for different compounds of a compound test set, wherein said in vitro data and said in vivo data correspond to one or more input variables of said equations, and optionally one or more output variables of said equations, and wherein said fitting generates one or more best fit values for said adjustment parameter; and

(iii) generating with said computer a selectively optimized value for said adjustment parameter of said simulation model by selecting one or more of said best fit values that, when assigned as an initial value to said adjustment parameter, permit said simulation model to predict an in vivo property of a compound in said mammalian system when in vitro data for said compound that corresponds to one or more input variables of said equations is utilized as input into said simulation model.

56. The method of claim 55, wherein said physiologic-based simulation model comprises a physiologic pharmacokinetic model of one or more anatomical segments of said mammalian system of interest.

57. The method of claim 56, wherein said physiologic pharmacokinetic model comprises differential equations for calculating the change in one or more physiological parameters of one or more of said anatomical segments and the movement and disposition of said compound in one or more of said anatomical segments as a function of time.

58. The method of claim 56, wherein said mammalian system of interest is selected from the group consisting of gastrointestinal tract, liver, heart, kidney, eye, nose, lung, skin and brain.

59. The method of claim 56, wherein said mammalian system of interest is human.

60. The method of claim 57, wherein one or more of said differential equations is for calculating a variable of a parameter corresponding to one or more in vivo properties of said compound selected from the group consisting of absorption, distribution, metabolism, elimination, and toxicity.

61. The method of claim 55, wherein said different compounds of said compound test set include compounds exhibiting a diverse range of in vivo properties in said mammalian system of interest.

62. The method of claim 61, wherein said in vivo properties are selected from the group consisting of permeability, solubility, dissolution, activity, metabolism, and toxicity.

63. The method of claim 55, wherein said in vivo data is derived from testing of a compound for an in vivo property in said mammalian system of interest.

64. The method of claim 55, wherein said in vitro data is derived from testing of a compound for an in vitro property in an assay that generates data selected from the group consisting of cell, tissue, physicochemical, structure-activity relationship (SAR) SAR, and quantitative structure-activity relationship (QSAR) QSAR data.

65. The method of claim 55, wherein said in vitro data and said in vivo data comprise a variable of a parameter corresponding to one or more in vitro and in vivo properties of said compound selected from the group consisting of absorption, distribution, metabolism, elimination, and toxicity.

66. The method of claim 55, wherein said fitting is simultaneous.

67. The method of claim 55, wherein said curve-fitting algorithm is a regression-based algorithm.

68. The method of claim 55, which further comprises reversibly storing said selectively optimized value for said adjustment parameter in a computer-implemented database.

69. The method of claim 55, which further comprises repeating steps (i) to (iii) one or more times for one or more additional adjustment parameters.

70. A computer-implemented method of generating a selectively optimized value for an adjustment parameter of a physiologic-based simulation model for predicting an in vivo property of a compound in a first mammalian system of interest from an in

vivo property of said compound in a second mammalian system of interest, said method comprising:

(i) providing a computer having as operably linked computer-implemented components a curve-fitting algorithm and a physiologic-based simulation model of a first mammalian system of interest, wherein said simulation model comprises one or more equations having an input variable for calculating as an output variable an in vivo property of a compound in said first mammalian system as a function of time, and wherein one or more of said equations is modified by an adjustment parameter;

(ii) using said computer and said curve-fitting algorithm, fitting with said simulation model a combination of in vivo data for different compounds of a compound test set derived from testing of said different compounds in said first mammalian system and in said second mammalian system, wherein said in vivo data corresponds to one or more input variables of said equations, and optionally one or more output variables of said equations, and wherein said fitting generates one or more best fit values for said adjustment parameter; and

(iii) generating with said computer a selectively optimized adjustment value for said adjustment parameter of said simulation model by selecting one or more of said best fit values that, when assigned as an initial value to said adjustment parameter, permit said simulation model to predict an in vivo property of a compound in said first mammalian system when in vivo data for said compound that is derived from testing of said compound in said second mammalian system and that corresponds to one or more input variables of said equations is utilized as input into said simulation model.

71. A computer-implemented method of selectively optimizing a physiologic-based simulation model for predicting an in vivo property of a compound in a mammalian system of interest from an in vitro property of said compound, said method comprising:

(i) providing a computer having as operably linked computer-implemented components a curve-fitting algorithm and a physiologic-based simulation model of a mammalian system of interest, said simulation model having equations for independent parameters comprising physiological parameters of said mammalian system and physicochemical parameters of a compound in said mammalian system, said equations having input variables for calculating as an output variable an in vivo property of a compound in said mammalian system as a function of time, and wherein one or more of said independent parameters is modified by an adjustment parameter;

(ii) using said computer and said curve-fitting algorithm, fitting with said simulation model a combination of in vitro data and in vivo data for different compounds of a compound test set, wherein said in vitro data and said in vivo data correspond to one or more input variables of said equations, and optionally one or more output variables of said equations, and wherein said fitting generates one or more best fit values for said adjustment parameter; and

(iii) generating with said computer a selectively optimized value for said adjustment parameter for one or more independent parameters of said simulation model by selecting one or more of said best fit values that, when assigned as an initial value to said adjustment parameter, permit said simulation model to predict an in vivo property of a compound in said mammalian system when in vitro data for said compound that corresponds to one or more input variables of said equations is utilized as input into said simulation model;

(iv) repeating steps (i) through (iii) one or more times for one or more additional independent parameters of said simulation model until deviation of predictability of said in vivo property of said compound in said mammalian system is minimized; and

(v) utilizing said selectively optimized values for said adjustment parameters as constants for said independent parameters in said simulation model for predicting said in vivo property of a compound in said mammalian system of interest.

72. A computer-implemented method for selectively optimizing a physiologic pharmacokinetic simulation model for predicting a pharmacokinetic property of a compound in a mammalian system of interest, said method comprising the steps of:

(i) providing a computer having as operably linked computer-implemented components a curve-fitting algorithm and a physiologic pharmacokinetic simulation model of a mammalian system of interest, wherein said simulation model comprises differential equations having input variables for calculating as an output variable a pharmacokinetic property of a compound in said mammalian system as a function of time, and wherein said equations include one or more independent parameters modified by an adjustment parameter,

(ii) using said computer and said curve-fitting algorithm, generating selectively optimized values for said adjustment parameter for one or more of said independent parameters by fitting with said simulation model a combination of data for different compounds of a compound test set, wherein said data correspond to one or more of said input variables, and optionally one or more of said output variables, and wherein said data is derived from (a) a first data source corresponding to the mammalian system of interest, and (b) a second data source corresponding to a system other than the mammalian system of interest;

(iii) selecting one or more best fit values for said adjustment parameter that, when assigned as an initial value for said adjustment parameter, permit correlation of one or more of said input variables from said first data source to one or more of said input variables from said second data source when using either or both of said first data source and said second data source as input variables in said simulation model to predict said pharmacokinetic property;

(iv) repeating steps (i) and (ii) one or more times for one or more additional independent parameters of said simulation model until deviation of predictability of said pharmacokinetic property when using either or both of said first data source and said second data source as input variables in said simulation model is minimized; and

(v) utilizing said selectively optimized values as constants for said adjustment parameters in said simulation model for predicting said pharmacokinetic property of a compound in said mammalian system of interest.

73. A computer-implemented method of generating a selectively optimized adjustment parameter of a physiologic pharmacokinetic simulation model of a mammalian system of interest for predicting a pharmacokinetic property of a compound in said mammalian system from in vitro data, said method comprising:

(i) providing a computer having as a computer implemented physiologic pharmacokinetic simulation model of a mammalian system of interest, said simulation model comprising equations having one or more input variables for calculating as one or more output variables a pharmacokinetic property of a compound in said mammalian system as a function of time, wherein one or more of said equations is modified by an adjustment parameter;

(ii) assigning an initial value to a selected adjustment parameter of said simulation model;

(iii) fitting a combination of in vitro data and in vivo data for different compounds of a compound test set with said simulation model utilizing a curve fitting algorithm that estimates the change required in said initial value in order to change one or more of said output variables;

(iv) selecting a best fit value for said selected adjustment parameter that, when assigned as an initial value to said selected adjustment parameter, permits said simulation model to predict said pharmacokinetic property of said compound when said input data comprises said in vitro data;

(v) assigning said best fit value as a constant to said selected adjustment parameter so as to generate said selectively optimized adjustment parameter that modifies one or more of said equations; and

(vi) optionally repeating steps (i) through (v) one or more times for one or more additional adjustment parameters.

74. The method of claim 73, wherein said in vitro data is obtained from testing of a compound in one or more assays that generate data selected from the group consisting of cell, tissue, structure-activity relationship (SAR), and quantitative structure-activity relationship (QSAR) data.

75. The method of claim 73, wherein said different compounds of a compound test set comprise compounds having diverse pharmacokinetic properties in said mammalian system of interest.

76. A computer-implemented method of generating a selectively optimized adjustment parameter of a physiologic pharmacokinetic simulation model of a mammalian system of interest that corresponds to a second species of mammal for

predicting a pharmacokinetic property of a compound in said mammalian system from in vivo data obtained from a first species of mammal, said method comprising:

(i) providing a computer having as a computer implemented physiologic pharmacokinetic simulation model of a mammalian system of interest, said simulation model comprising equations having one or more input variables for calculating as one or more output variables a pharmacokinetic property of a compound in said mammalian system as a function of time, wherein one or more of said equations is modified by an adjustment parameter;

(ii) assigning an initial value to a selected adjustment parameter of said simulation model;

(iii) fitting a combination of in vivo data with said simulation model, said combination of in vivo data derived from testing of different compounds of a compound test set in said first species of mammal and said second species of mammal, and said fitting is performed utilizing a curve fitting algorithm that estimates the change required in said initial value in order to change one or more of said input variables, and optionally one or more of said output variables;

(iv) selecting a best fit value for said selected adjustment parameter that, when assigned as an initial value to said selected adjustment parameter, permits said simulation model to predict said pharmacokinetic property of said compound when said input data comprises said in vivo data from said first species of mammal;

(v) assigning said best fit value as a constant to said selected adjustment parameter so as to generate said selectively optimized adjustment parameter that modifies one or more of said equations; and

(vi) optionally repeating steps (i) through (v) one or more times for one or more additional adjustment parameters.

77. The method of claim 76, wherein said different compounds of a compound test set comprise compounds having diverse pharmacokinetic properties in said mammalian system of interest.

78. The method of claim 73 or 76, wherein said selectively optimized adjustment parameter is for one or more of fluid absorption, flux, permeability, transport mechanism, transfer rate, dissolution, solubility and segment surface area.

79. The method of claim 73 or 76, which further comprises reversibly storing said constant for said selectively optimized adjustment parameter in a computer-implemented database.

80. A computer system configured to predict a pharmacokinetic property of a target compound in a target anatomical segment of a target mammalian system from a pharmacokinetic property of a test compound in an anatomical segment of a data source system, said computer comprising as operably linked components:

(a) an input/output system,

(b) a simulation engine, and

(c) a stored physiologic pharmacokinetic simulation model of said target mammalian system, said simulation model comprising:

(i) differential equations for calculating a change in one or more physiological parameters of said target mammalian system and the movement and disposition of said target compound in said target mammalian system as a function of time, using input data for said differential equations comprising the pharmacokinetic property of the test compound in the anatomical segment of said data source system; and

(ii) boundary condition parameter values for said differential equations corresponding to parameters of said target mammalian system, and

(iii) a logic function module having control statement rules for initiating said physiologic pharmacokinetic simulation model of said mammalian system function.

81. The computer system of claim 80 wherein said computer, using the model, generates estimated values for a selected pharmacokinetic property of said target compound when supplied with input values corresponding to said selected pharmacokinetic property of said test compound in a portion of said data source system by the method comprising:

(a) entering into said input/output system input data comprising a pharmacokinetic property of said test compound in a segment of said data source system; and

(b) applying said simulation engine and said simulation model, and invoking said simulation engine model for predicting said pharmacokinetic property of said target compound in a segment of said target mammalian system.

82. A computer system for simulating absorption of a compound in a mammal, said system having as computer-implemented components an input/output system, simulation engine, and simulation model of one or more segments of a selected mammalian system having one or more physiological barriers to absorption based on a selected route of administration, said simulation model comprising as operably linked components:

(i) differential equations for one or more of fluid transit, fluid absorption, mass transit, mass dissolution, mass solubility, and mass absorption for one or more segments of said mammalian system; (ii) initial parameter values for said differential equations corresponding to physiological parameters and selectively optimized adjustment parameters, and optionally regional correlation parameters, for one or more segments of said mammalian system; and (iii) control statement rules for one or more of transit, absorption, permeability, solubility, dissolution, and concentration for one or more segments of said mammalian system;

said input/output system, said simulation engine, and said simulation model being capable of working together to carry out the steps of:

(a) receiving as input data through said input/output system, dose, permeability and solubility data for said compound for one or more segments of said mammalian system; and

(b) applying said simulation engine and said simulation model to simulate absorption of said compound relative to one or more segments of said mammalian system.

83. A computer system for simulating a pharmacokinetic property of a compound in a mammalian system of interest, said computer system comprising as operably linked computer-implemented components an input/output system, a simulation engine, and a physiologic pharmacokinetic simulation model of one or more anatomical segments of said mammalian system of interest, said simulation model comprising differential equations for calculating as a function of time the change in (i) a physiological parameter of one or more of said segments and (ii) a pharmacokinetic property comprising an absorption parameter of a compound relative to a selected route of administration, barrier to absorption and sampling site of one or more of said segments, wherein one or more of said differential equations is modified by a selectively optimized adjustment parameter;

said input/output system, said simulation engine, and said physiologic pharmacokinetic simulation model being capable of working together to carry out the steps of:

(a) receiving through said computer readable input/output system input data comprising dose, permeability and solubility data for said compound for one or more segments of said mammalian system of interest; and

(b) applying said simulation engine and said physiologic pharmacokinetic simulation model to simulate a pharmacokinetic property of said compound relative to one or more segments of said mammalian system of interest.

84. The computer system of claim 83, wherein said differential equations are for one or more of fluid transit, fluid absorption, mass transit, mass dissolution, mass solubility, and mass absorption for one or more segments of said mammalian system.

85. The computer system of claim 83, wherein said differential equations comprise initial parameter values corresponding to said physiological parameter and said selectively optimized adjustment parameter for one or more segments of said mammalian system.

86. The computer system of claim 83, wherein said physiologic pharmacokinetic simulation model comprises control statement rules for one or more of transit,

absorption, permeability, solubility, dissolution, and concentration for one or more segments of said mammalian system.

87. The computer system of claim 86, wherein said control statement rules are IF...THEN production rules.

88. The computer system of claim 83, wherein said input/output system comprises a user interface.

89. The computer system of claim 83, wherein said simulation engine comprises a differential equation solver.

90. The computer system of claim 83, wherein said pharmacokinetic property is selected from the group consisting of absorption, distribution, metabolism, elimination and toxicity.

91. The computer system of claim 83, wherein said absorption parameter is selected from the group consisting of concentration, permeability, solubility, dissolution rate, transport mechanism, and formulation release rate.

92. The article of manufacture of claim 83, wherein said physiological parameter is selected from the group consisting of pH, fluid volume, fluid volume transfer rate, fluid absorption, surface area, and transit time.

93. The computer system of claim 83, wherein said mammalian system of interest is selected from the group consisting of gastrointestinal tract, liver, heart, kidney, eye, nose, lung, skin and brain.

94. The computer system of claim 93, wherein said mammalian system of interest is gastrointestinal tract and said segments are selected from the group consisting of stomach, duodenum, jejunum, ileum and colon.

95. The computer system of claim 83, wherein said input data includes data selected from the group consisting of dissolution rate, transport mechanism and formulation release rate.

96. The computer system of claim 83, wherein said differential equations comprise one or more input variables corresponding to said input data for calculating as one or more output variables said change in said physiological parameter.

97. The computer system of claim 83, wherein said differential equations comprise one or more input variables corresponding to said input data for calculating as one or more output variables said change in said absorption parameter.

98. The computer system of claim 83, wherein said selectively optimized adjustment parameter correlates said input data to output data comprising said pharmacokinetic property of said compound.

99. The computer system of claim 98, wherein said input data comprises in vitro data and said selectively optimized adjustment parameter comprises a best fit value obtained by (i) assigning an initial value to a selected adjustment parameter of said simulation model, (ii) fitting a combination of in vitro data and in vivo data for different compounds of a compound test set with said simulation model, (iii) selecting a best fit value for said selected adjustment parameter that, when assigned as an initial value to said selected adjustment parameter, permits said simulation model to predict said pharmacokinetic property of said compound when said input data comprises said in vitro data, and (iv) assigning said best fit value as a constant to said selected adjustment parameter so as to generate said selectively optimized adjustment parameter that modifies one or more of said differential equations.

100. The computer system of claim 99, wherein said in vitro data is obtained from testing of a compound in one or more assays that generate data selected from the group consisting of cell, tissue, structure-activity relationship (SAR), and quantitative structure-activity relationship (QSAR) data.

101. The computer system of claim 99, wherein said different compounds of a compound test set comprise compounds having diverse pharmacokinetic properties in said mammalian system of interest.

102. The computer system of claim 98, wherein said input data comprises in vivo data from a first species of mammal and said mammalian system of interest comprises a second species of mammal, and wherein said selectively optimized adjustment

parameter comprises a best fit value obtained by (i) assigning an initial value to a selected adjustment parameter of said simulation model, (ii) fitting a combination of in vivo data with said simulation model, said combination of in vivo data derived from testing of different compounds of a compound test set in said first species of mammal and said second species of mammal, (iii) selecting a best fit value for said selected adjustment parameter that, when assigned as an initial value to said selected adjustment parameter, permits said simulation model to predict said pharmacokinetic property of said compound when said input data comprises said in vitro data from said first species of mammal, and (iv) assigning said best fit value as a constant to said selected adjustment parameter so as to generate said selectively optimized adjustment parameter that modifies one or more of said differential equations.

103. The computer system of claim 102, wherein said different compounds of a compound test set comprise compounds having diverse pharmacokinetic properties in said mammalian system of interest.

104. The computer system of claim 83, wherein said selectively optimized adjustment parameter is for one or more of fluid absorption, flux, permeability, transport mechanism, transfer rate, and segment surface area.

105. The computer system of claim 103, wherein said physiologic pharmacokinetic simulation model comprises at least two of said anatomical segments and a logic function module comprising a regional correlation estimation function and a control statement for initiating said function, wherein said estimation function when initiated is capable of generating an estimated value for a selected pharmacokinetic property of said compound in a first anatomical segment when supplied with an input value corresponding to said selected pharmacokinetic property in a second anatomical segment and with a regional correlation coefficient for said selected pharmacokinetic property of said first and second anatomical segments.

106. A computer system for simulating a pharmacokinetic property of a compound in a mammal of interest utilizing regional correlation parameter estimation, said computer system comprising as operably linked computer-implemented components an input/output system, a simulation engine, and a physiologic pharmacokinetic simulation model of at least two segments of a selected mammalian system of interest,

said physiologic pharmacokinetic simulation model comprising (i) differential equations for calculating the change in one or more physiological parameters of first and second segments of said mammalian system of interest and the movement and disposition of said compound in said first and second segments as a function of time, and (ii) a logic function module having a regional correlation parameter estimation function and a control statement for initiating said function, wherein said estimation function when initiated is capable of generating an estimated value for a selected pharmacokinetic property comprising an absorption parameter of said compound in said first segment when supplied with an input value corresponding to said selected pharmacokinetic property of said compound in said second segment and with a regional correlation coefficient for said selected pharmacokinetic parameter of said first and second segments;

said input/output system, said simulation engine, and said physiologic pharmacokinetic simulation model being capable of working together to carry out the steps of:

(a) receiving through said input/output system input data comprising a pharmacokinetic property of said compound in said second segment of said mammalian system of interest; and

(b) applying said simulation engine and said physiologic pharmacokinetic simulation model to initiate said estimation function to estimate said pharmacokinetic property of said compound in said first segment of said mammalian system of interest.

107. The computer system of claim 106, wherein said regional correlation estimation function comprises a function/transformation algorithm.

108. The computer system of claim 107, wherein said function/transformation algorithm is selected from the group consisting of a polynomial, exponential, and logarithm.

109. The computer system of claim 106, wherein said regional correlation coefficient comprises a best fit value that transforms said input data comprising said pharmacokinetic property of said compound in said second segment to an estimated pharmacokinetic property of said compound in said first segment.

110. The computer system of claim 106, wherein said pharmacokinetic property is selected from the group consisting of absorption, distribution, metabolism, elimination and toxicity.

111. The computer system of claim 106, wherein said pharmacokinetic parameter is selected from the group consisting of permeability, solubility, dissolution rate and transport mechanism.

112. The computer system of claim 106, wherein said differential equations are selected from the group consisting of equations for fluid transit, fluid absorption, mass transit, mass dissolution, mass solubility, and mass absorption.

113. The computer system of claim 106, wherein said mammalian system of interest is selected from the group consisting of gastrointestinal tract, liver, heart, kidney, eye, nose, lung, skin and brain.

114. The computer system of claim 83 or 106, wherein said mammalian system of interest is human.

115. The computer system of claim 106, wherein said input data comprises in vitro data.

116. The computer system of claim 115, wherein said in vitro data is derived from testing of said compound in an assay that generates data selected from the group consisting of cell, tissue, physicochemical, structure-activity relationship (SAR) SAR, and quantitative structure-activity relationship (QSAR) QSAR data.

117. The computer system of claim 106, wherein said computer system comprises a data processor, a memory and a display.

118. The computer system of claim 106, wherein said input/output system comprises a user interface.

119. The computer system of claim 106, wherein said simulation engine comprises a differential equation solver.

120. The computer system of claim 106, wherein said physiologic pharmacokinetic simulation model comprises a subsystem of said computer system.

121. The computer system of claim 106, wherein one or more of said differential equations is modified by a selectively optimized adjustment parameter.

122. A subsystem for use in a computer system for simulating oral absorption of a compound in a mammal, said subsystem comprising:

(i) a computer-implemented simulation model of one or more segments of the gastrointestinal (GI) track of a mammal comprising differential equations for one or more of fluid transit, fluid absorption, mass transit, mass dissolution, mass solubility, and mass absorption for one or more segments of the GI tract of said mammal; and

(ii) a computer-implemented database comprising initial parameter values for said differential equations corresponding to physiological parameters and selectively optimized adjustment parameters, and optionally regional correlation parameters, for one or more segments of the GI tract of said mammal.

123. A computer-implemented database according to claim 122 having a compartment-flow model data structure.

124. A subsystem for use in a computer system for simulating oral absorption of a compound in a mammal, said subsystem comprising:

(i) a computer-implemented simulation model of one or more segments of the gastrointestinal (GI) track of a mammal comprising differential equations for one or more of fluid transit, fluid absorption, mass transit, mass dissolution, mass solubility, and mass absorption for one or more segments of the GI tract of said mammal; and

(ii) a computer-implemented database comprising initial parameter values for said differential equations corresponding to physiological parameters and regional correlation parameters for one or more segments of the GI tract of said mammal.

125. A computer-implemented database according to claim 124 having a compartment-flow model data structure.

126. The subsystem of claim 122 or 124, wherein said computer-implemented database comprises computer-implemented control statement rules for one or more of

transit, absorption, permeability, solubility, dissolution, and concentration for one or more segments of the GI tract of said mammal.

127. A computer-implemented database for use in a computer system for simulating absorption of a compound in a mammal, said computer-implemented database comprising:

a computer-implemented physiologic-based simulation model of one or more segments of selected mammalian system of interest comprising (i) differential equations for one or more of fluid transit time, fluid absorption, mass transit time, mass dissolution, mass solubility, and mass absorption for one or more segments of said mammalian system; (ii) initial parameter values for said differential equations corresponding to physiological parameters and adjustment parameters, and optionally one or more regional correlation parameters, for one or more segments of said mammalian system; and (iii) control statement rules for one or more of transit time, absorption, permeability, dissolution, concentration, and mathematical error correction for one or more segments of said mammalian system;

wherein said computer-implemented physiologic-based simulation model comprises a compartment-flow data structure for calculating time-dependent rate of absorption, extent of absorption, and concentration of a compound at a sampling site across a physiological barrier of one or more segments of said mammalian system when applied in a simulation engine having a differential equation solver and a control statement module.

128. The computer-implemented database of claim 127, wherein said adjustment parameters are selected from the group consisting of regional fluid absorption, permeability, flux, active transport, carrier mediated transport, compound efflux, transfer rate, and surface area.

129. The computer-implemented database of claim 127, wherein said physiological parameters are selected from the group consisting of soluble mass transfer rate constant, permeability, solubility, dissolution rate, transport mechanism, pH, initial volume, surface area, transit time, fluid volume transfer rate, and fluid absorption rate.

130. The computer-implemented database of claim 127, wherein said regional correlation parameters are for permeability.

131. A computer-implemented physiological simulation model of the gastrointestinal (GI) track of a mammal for simulating oral absorption of a compound in said mammal, said physiological simulation model corresponding to a compartment-flow model comprising:

compartments characterized by fluid volume, fluid absorption, insoluble mass, soluble mass, and mass absorption for one or more of segments of the GI track of a mammal, wherein said compartments are operably linked through flow regulators and converters, wherein said flow regulators regulate flow among compartments and said converters modify said flow regulators, and wherein said flow regulators are characterized by fluid absorption rate, fluid transit rate, insoluble mass transit rate, insoluble mass dissolution rate, soluble mass transit rate, and soluble mass absorption rate.

132. The computer-implemented physiological simulation model of claim 131, wherein said converters are characterized by fluid volume, fluid volume absorption rate constant, fluid volume transit rate constant, insoluble mass, insoluble mass transit rate constant, dissolution rate constant, soluble mass, soluble mass transit rate constant, surface area, dissolved mass concentration and permeability.

133. The computer-implemented physiological simulation model of claim 131, which further comprises compartments characterized by formulation and flow regulators characterized by formulation transit rate and formulation release rate.

134. A computer-implemented gastrointestinal (GI) transit simulation model for simulating mass and fluid loss in the GI track of a mammal, said GI transit simulation model corresponding to a compartment-flow model comprising:

compartments characterized by fluid volume and fluid volume absorption for stomach, duodenum, jejunum, ileum and colon that are operably linked through flow regulators and one or more converters that modify one or more of said flow regulators, wherein said flow regulators are characterized by fluid volume absorption rate and fluid volume transit rate, and wherein said converters are characterized by selectively optimized adjustment parameter values for one or more of fluid absorption rate constant and fluid volume transit rate constant.

135. A computer-implemented solubility simulation model for simulating pH dependent solubility and dissolution of a compound in the gastrointestinal (GI) track of a mammal, said solubility simulation model corresponding to a compartment-flow model comprising:

compartments characterized by insoluble mass and soluble mass for stomach, duodenum, jejunum, ileum and colon that are operably linked through flow regulators and one or more converters that modify one or more of said flow regulators, wherein said flow regulators are characterized by insoluble mass transit rate, insoluble mass dissolution rate, and soluble mass transit rate, wherein said converters are characterized by insoluble mass, insoluble mass transit rate constant, insoluble mass dissolution rate constant, soluble mass, and soluble mass transit rate constant, and wherein one or more of said converters are characterized by selectively optimized adjustment parameters.

136. A computer-implemented absorption simulation model for simulating absorption of a compound in the gastrointestinal (GI) track of a mammal to at least the portal vein, said absorption simulation model corresponding to a compartment-flow model comprising:

compartments characterized by soluble mass and soluble mass absorption for stomach, duodenum, jejunum, ileum and colon that are operably linked through flow regulators and one or more converters that modify said flow regulators, where said flow regulators are characterized by insoluble mass transit rate, insoluble mass dissolution rate, soluble mass transit rate, soluble mass absorption rate, where said converters are characterized by insoluble mass, insoluble mass dissolution rate constant, soluble mass transit rate constant, surface area, dissolved mass concentration, and permeability, and wherein one or more of said converters are characterized by selectively optimized adjustment parameters.

137. An article of manufacture comprising a computer readable medium having computer readable program code embodied therein for simulating absorption of a target compound in a mammal of interest, and having

(a) computer readable simulation engine program code, and

(b) computer readable simulation model code of one or more segments of a selected mammalian system having one or more physiological barriers to absorption of said target compound, said computer readable simulation model comprising as operably linked components:

(i) differential equations for one or more of fluid transit, fluid absorption, mass transit, mass dissolution, mass solubility, and mass absorption for one or more segments of said mammalian system, and

(ii) control statement rules for one or more segments of said mammalian system;

said computer readable simulation engine code and simulation model code being being configured to carry out the steps of:

(a) receiving input data for a compound for one or more segments of a data source system; and

(b) applying said computer readable simulation engine code and said computer readable simulation model code to simulate absorption of said target compound relative to one or more segments of said target mammalian system.

138. An article of manufacture comprising a computer readable medium having computer readable program code embodied therein for simulating absorption of a compound in a mammal of interest, and having computer readable input/output system, computer readable simulation engine, and computer readable simulation model of one or more segments of a selected mammalian system having one or more physiological barriers to absorption of said compound based on a selected route of administration, said computer readable simulation model comprising as operably linked components:

(i) differential equations for one or more of fluid transit, fluid absorption, mass transit, mass dissolution, mass solubility, and mass absorption for one or more segments of said mammalian system; (ii) initial parameter values for said differential equations corresponding to physiological parameters and selectively optimized adjustment parameters, and optionally one or more regional correlation parameters, for one or more segments of said mammalian system; and optionally (iii) control statement rules for one or more of transit, absorption, permeability, solubility, dissolution, and concentration for one or more segments of said mammalian system;

said computer readable input/output system, said computer readable simulation engine, and said computer readable simulation model being capable of working together to carry out the steps of:

(a) receiving as input data through said computer readable input/output system, dose, permeability and solubility data for said compound for one or more segments of said mammalian system; and

(b) applying said computer readable simulation engine and said computer readable simulation model to simulate absorption of said compound relative to one or more segments of said mammalian system of interest.

139. The article of manufacture of claim138, wherein said mammalian system is selected from the group consisting of the gastrointestinal tract, the eye, the nose, the lung, the skin, and the brain.

140. The article of manufacture of claim 139, wherein said mammalian system is the gastrointestinal tract.

141. The article of manufacture of claim 140, wherein said segments are selected from the group consisting of stomach, duodenum, jejunum, ileum, and colon.

142. The article of manufacture of claim 138, wherein said simulation model corresponds to a compartment-flow model comprising compartments that are operably linked through flow regulators modified by one or more converters.

143. The article of manufacture of claim 142, wherein said compartments comprise one or more compartments characterized by a parameter selected from the group consisting of fluid volume, fluid absorption, formulation, insoluble mass, soluble mass, and soluble mass absorption.

144. The article of manufacture of claim 142, wherein said flow regulators are characterized by a parameter selected from the group consisting of fluid absorption

rate, fluid transit rate, formulation transit rate, formulation release rate, insoluble mass transit rate, insoluble mass dissolution rate, soluble mass transit rate, and soluble mass absorption rate.

145. The article of manufacture of claim 142, wherein said converters are characterized by a parameter selected from the group consisting of fluid volume, fluid volume absorption rate constant, fluid volume transit rate constant, insoluble mass, insoluble mass transit rate constant, dissolution rate constant, soluble mass, soluble mass transit rate constant, surface area, dissolved mass concentration and permeability.

146. The article of manufacture of claim 142, wherein one or more of said converters are characterized by a selectively optimized adjustment parameter.

147. The article of manufacture of claim 142, wherein one or more of said converters are characterized by a regional correlation parameter.

148. The article of manufacture of claim 138, wherein said control statement rules are IF...THEN production rules.

149. The article of manufacture of claim 138, wherein said physiological parameters are characterized by a parameter selected from the group consisting of soluble mass transfer rate constant, permeability, solubility, dissolution rate, and transport mechanism.

150. The article of manufacture of claim 138, wherein said physiological parameters are characterized by a parameter selected from the group consisting of pH, initial fluid volume, surface area, fluid volume transit time, insoluble mass transit time, soluble mass transit time, fluid volume transfer rate, and fluid absorption rate.

151. The article of manufacture of claim 138, wherein said mammal is human.

152. The article of manufacture of claim 138, where said input data further comprises data for said compound of interest selected from the group consisting of dissolution rate, transport mechanism, transit time, pH and formulation release rate.

153. The article of manufacture of claim 138, wherein said input data is *in vitro* data.

154. The article of manufacture of claim 153, wherein said *in vitro* data is permeability data derived from an assay selected from the group consisting of a cell-based assay and a tissue-based assay.

155. The article of manufacture of claim 153, wherein said *in vitro* data is transport mechanism data derived from an assay selected from the group consisting of a cell-based assay and a tissue-based assay.

156. The article of manufacture of claim 153, wherein said *in vitro* data is permeability data derived from structure-activity relationship data of said compound.

157. The article of manufacture of claim 153, wherein said *in vitro* data is dissolution rate data derived from structure-activity relationship data of said compound.

158. The article of manufacture of claim 153, wherein said *in vitro* data is solubility data derived from structure-activity relationship data of said compound.

159. A computer program product for simulating absorption of a compound in a mammal, and having computer readable program code input/output system, computer readable program code simulation engine, and computer readable program code simulation model of one or more segments of a selected mammalian system having one or more physiological barriers to absorption based on a selected route of administration, said computer readable program code simulation model comprising as operably linked components:

(i) differential equations for one or more of fluid transit, fluid absorption, mass transit, mass dissolution, mass solubility, and mass absorption for one or more segments of said mammalian system; (ii) initial parameter values for said differential equations corresponding to physiological parameters and selectively optimized adjustment parameters, and optionally regional correlation parameters, for one or more segments of said mammalian system; and (iii) control statement rules for one or more of transit, absorption, permeability, solubility, dissolution, and concentration for one or more segments of said mammalian system;

wherein said computer readable program code input/output system, said computer readable program code simulation engine, and said computer readable program code simulation model being capable of working together to carry out the steps of:

(a) receiving as input data through said computer readable program code input/output system, dose, permeability and solubility data for said compound for one or more segments of said mammalian system; and

(b) applying said computer readable program code simulation engine and said computer readable program code simulation model to simulate absorption of said compound relative to one or more segments of said mammalian system.

160. An article of manufacture comprising a computer readable medium having computer readable program code embodied therein for simulating a pharmacokinetic property of a compound in a mammal of interest, and having computer readable input/output system, computer readable simulation engine, and computer readable physiologic pharmacokinetic simulation model of one or more anatomical segments of a selected mammalian system, said computer readable physiologic pharmacokinetic simulation model comprising differential equations for calculating as a function of time the change in (i) a physiological parameter of one or more of said segments and (ii) a pharmacokinetic property comprising an absorption parameter of a compound relative to a selected route of administration, barrier to absorption and sampling site of one or more of said segments, wherein one or more of said differential equations is modified by a selectively optimized adjustment parameter;

said computer readable input/output system, said computer readable simulation engine, and said computer readable physiologic pharmacokinetic simulation model being capable of working together to carry out the steps of:

(a) receiving through said computer readable input/output system input data comprising dose, permeability and solubility data for said compound for one or more segments of said mammalian system of interest; and

(b) applying said computer readable simulation engine and said computer readable physiologic pharmacokinetic simulation model to simulate a pharmacokinetic property of said compound relative to one or more segments of said mammalian system of interest.

161. The article of manufacture of claim 160, wherein said differential equations are for one or more of fluid transit, fluid absorption, mass transit, mass dissolution, mass solubility, and mass absorption for one or more segments of said mammalian system.

162. The article of manufacture of claim 160, wherein said differential equations comprise initial parameter values corresponding to said physiological parameter and said selectively optimized adjustment parameter for one or more segments of said mammalian system.

163. The article of manufacture of claim 160, wherein said physiologic pharmacokinetic simulation model comprises control statement rules for one or more of transit, absorption, permeability, solubility, dissolution, and concentration for one or more segments of said mammalian system.

164. The article of manufacture of claim 163, wherein said control statement rules are IF...THEN production rules.

165. The article of manufacture of claim 160, wherein said input/output system comprises a user interface.

166. The article of manufacture of claim 160, wherein said simulation engine comprises a differential equation solver.

167. The article of manufacture of claim 160, wherein said absorption parameter is selected from the group consisting of concentration, permeability, solubility, dissolution rate, transport mechanism, and formulation release rate.

168. The article of manufacture of claim 160, wherein said physiological parameter is selected from the group consisting of pH, fluid volume, fluid volume transfer rate, fluid absorption, surface area, and transit time.

169. The article of manufacture of claim 160, wherein said mammalian system of interest is selected from the group consisting of gastrointestinal tract, liver, heart, kidney, eye, nose, lung, skin and brain.

170. The article of manufacture of claim 169, wherein said mammalian system of interest is gastrointestinal tract and said segments are selected from the group consisting of stomach, duodenum, jejunum, ileum and colon.

171. The article of manufacture of claim 160, wherein said input data includes data selected from the group consisting of dissolution rate, transport mechanism and formulation release rate.

172. The article of manufacture of claim 160, wherein said differential equations comprise one or more input variables corresponding to said input data for calculating as one or more output variables said change in said physiological parameter.

173. The article of manufacture of claim 160, wherein said differential equations comprise one or more input variables corresponding to said input data for calculating as one or more output variables said change in said absorption parameter.

174. The article of manufacture of claim 160, wherein said selectively optimized adjustment parameter correlates said input data to output data comprising said pharmacokinetic property of said compound.

175. The article of manufacture of claim 174, wherein said input data comprises *in vitro* data and said selectively optimized adjustment parameter comprises a best fit value obtained by (i) assigning an initial value to a selected adjustment parameter of said simulation model, (ii) fitting a combination of *in vitro* data and *in vivo* data for different compounds of a compound test set with said simulation model, (iii) selecting a best fit value for said selected adjustment parameter that, when assigned as an initial value to said selected adjustment parameter, permits said simulation model to predict said pharmacokinetic property of said compound when said input data comprises said *in vitro* data, and (iv) assigning said best fit value as a constant to said selected adjustment parameter so as to generate said selectively optimized adjustment parameter that modifies one or more of said differential equations.

176. The article of manufacture of claim 175, wherein said *in vitro* data is obtained from testing of a compound in one or more assays that generate data selected from the group consisting of cell, tissue, structure-activity relationship (SAR), and quantitative structure-activity relationship (QSAR) data.

177. The article of manufacture of claim 175, wherein said different compounds of a compound test set comprise compounds having diverse pharmacokinetic properties in said mammalian system of interest.

178. The article of manufacture of claim 174, wherein said input data comprises *in vivo* data from a first species of mammal and said mammalian system of interest comprises a second species of mammal, and wherein said selectively optimized adjustment parameter comprises a best fit value obtained by (i) assigning an initial value to a selected adjustment parameter of said simulation model, (ii) fitting a combination of *in vivo* data with said simulation model, said combination of *in vivo* data derived from testing of different compounds of a compound test set in said first species of mammal and said second species of mammal, (iii) selecting a best fit value for said selected adjustment parameter that, when assigned as an initial value to said selected adjustment parameter, permits said simulation model to predict said pharmacokinetic property of said compound when said input data comprises said *in vitro* data from said first species of mammal, and (iv) assigning said best fit value as a constant to said selected adjustment parameter so as to generate said selectively optimized adjustment parameter that modifies one or more of said differential equations.

179. The article of manufacture of claim 178, wherein said different compounds of a compound test set comprise compounds having diverse pharmacokinetic properties in said mammalian system of interest.

180. The article of manufacture of claim 160, wherein said selectively optimized adjustment parameter is for one or more of fluid absorption, flux, permeability, transport mechanism, transfer rate, and segment surface area.

181. The article of manufacture of claim 160, wherein said computer readable physiologic pharmacokinetic simulation model comprises at least two of said anatomical segments and a logic function module comprising a regional correlation

estimation function and a control statement for initiating said function, wherein said estimation function when initiated is capable of generating an estimated value for a selected pharmacokinetic property of said compound in a first anatomical segment when supplied with an input value corresponding to said selected pharmacokinetic property in a second anatomical segment and with a regional correlation coefficient for said selected pharmacokinetic property of said first and second anatomical segments.

182. A computer program product for simulating a pharmacokinetic property of a compound in a mammal of interest, and having computer readable program code input/output system, computer readable program code simulation engine, and computer readable program code physiologic pharmacokinetic simulation model of one or more anatomical segments of a selected mammalian system, said computer readable program code physiologic pharmacokinetic simulation model comprising differential equations for calculating as a function of time the change in (i) a physiological parameter of one or more of said segments and (ii) a pharmacokinetic property comprising an absorption parameter of a compound relative to a selected route of administration, barrier to absorption and sampling site of one or more of said segments, wherein one or more of said differential equations is modified by a selectively optimized adjustment parameter;

said computer readable program code input/output system, said computer readable program code simulation engine, and said computer readable program code physiologic pharmacokinetic simulation model being capable of working together to carry out the steps of:

(a) receiving through said computer readable program code input/output system input data comprising dose, permeability and solubility data for said compound for one or more segments of said mammalian system of interest; and

(b) applying said computer readable program code simulation engine and said computer readable program code physiologic pharmacokinetic simulation model to simulate a pharmacokinetic property comprising an absorption parameter of said compound relative to one or more segments of said mammalian system of interest.

183. The article of manufacture of claim 182, wherein said computer readable program code physiologic pharmacokinetic simulation model comprises at least two of said anatomical segments and a logic function model comprising a regional correlation estimation function and a control statement for initiating said function, wherein said estimation function when initiated is capable of generating an estimated value for a selected pharmacokinetic property of said compound in a first anatomical segment when supplied with an input value corresponding to said selected pharmacokinetic property in a second anatomical segment and with a regional correlation coefficient for said selected pharmacokinetic property of said first and second anatomical segments.

184. An article of manufacture comprising a computer readable medium having computer readable program code embodied therein for simulating a pharmacokinetic property of a compound in a mammal of interest, and having computer readable input/output system, computer readable simulation engine, and computer readable physiologic pharmacokinetic simulation model of at least two segments of a selected mammalian system of interest, said computer readable physiologic pharmacokinetic simulation model comprising as operably linked components:

(i) differential equations for calculating the change in one or more physiological parameters of first and second segments of said mammalian system of interest and the movement and disposition of said compound in said first and second segments as a function of time, and (ii) a logic function module having a regional correlation parameter estimation function and a control statement for initiating said function, wherein said estimation function when initiated is capable of generating an estimated value for a selected pharmacokinetic property comprising an absorption parameter of said compound in said first segment when supplied with an input value corresponding to said selected pharmacokinetic property of said compound in said second segment and with a regional correlation coefficient for said selected pharmacokinetic parameter of said first and second segments;

said computer readable input/output system, said computer readable simulation engine, and said computer readable physiologic pharmacokinetic simulation model being capable of working together to carry out the steps of:

(a) receiving through said computer readable input/output system input data comprising a pharmacokinetic property of said compound in said second segment of said mammalian system of interest; and

(b) applying said computer readable simulation engine and said computer readable physiologic pharmacokinetic simulation model to initiate said estimation function to estimate said pharmacokinetic property of said compound in said first segment of said mammalian system of interest.

185. The article of manufacture of claim 184, wherein said regional correlation estimation function comprises a function/transformation algorithm.

186. The article of manufacture of claim 185, wherein said function/transformation algorithm is selected from the group consisting of a polynomial, exponential, and logarithm.

187. The article of manufacture of claim 184, wherein said regional correlation coefficient comprises a best fit value that transforms said input data comprising said pharmacokinetic property of said compound in said second segment to an estimated pharmacokinetic property of said compound in said first segment.

188. The article of manufacture of claim 160 or 184, wherein said pharmacokinetic property is selected from the group consisting of absorption, distribution, metabolism, elimination and toxicity.

189. The article of manufacture of claim 184, wherein said pharmacokinetic parameter is selected from the group consisting of permeability, solubility, dissolution rate and transport mechanism.

190. The article of manufacture of claim 184, wherein said differential equations are selected from the group consisting of equations for fluid transit, fluid absorption, mass transit, mass dissolution, mass solubility, and mass absorption.

191. The article of manufacture of claim 184, wherein said mammalian system of interest is selected from the group consisting of gastrointestinal tract, liver, heart, kidney, eye, nose, lung, skin and brain.

192. The article of manufacture of claim 160 or 184, wherein said mammalian system of interest is human.

193. The article of manufacture of claim 184, wherein said input data comprises *in vitro* data.

194. The article of manufacture of claim 193, wherein said *in vitro* data is derived from testing of said compound in an assay that generates data selected from the group consisting of cell, tissue, physicochemical, structure-activity relationship (SAR) SAR, and quantitative structure-activity relationship (QSAR) QSAR data.

195. The article of manufacture of claim 184, wherein one or more of said differential equations is modified by a selectively optimized adjustment parameter.

196. A computer program product for simulating a pharmacokinetic property of a compound in a mammal of interest, and having computer readable program code input/output system, computer readable program code simulation engine, and computer readable program code physiologic pharmacokinetic simulation model of at least two segments of a selected mammalian system of interest, said computer readable program code physiologic pharmacokinetic simulation model comprising as operably linked components:

(i) differential equations for calculating the change in one or more physiological parameters of first and second segments of said mammalian system of interest and the movement and disposition of said compound in said first and second segments as a function of time, and (ii) a logic function module having a regional correlation parameter estimation function and a control statement for initiating said function, wherein said estimation function when initiated is capable of generating an estimated value for a selected pharmacokinetic property comprising an absorption parameter of said compound in said first segment when supplied with an input value corresponding to said selected pharmacokinetic property of said compound in said second segment and with a regional correlation coefficient for said selected pharmacokinetic parameter of said first and second segments;

said computer readable program code input/output system, said computer readable program code simulation engine, and said computer readable program code physiologic pharmacokinetic simulation model being capable of working together to carry out the steps of:

(a) receiving through said computer readable program code input/output system input data comprising a pharmacokinetic property of said compound in said second segment of said mammalian system of interest; and

(b) applying said computer readable program code simulation engine and said computer readable program code physiologic pharmacokinetic simulation model to initiate said estimation function to estimate said pharmacokinetic property of said compound in said first segment of said mammalian system of interest.

197. The computer program product of claim 196, wherein one or more of said differential equations is modified by a selectively optimized adjustment parameter.

198. An article of manufacture comprising a computer readable medium having embodied therein a computer readable physiologic pharmacokinetic simulation model of one or more anatomical segments of a selected mammalian system, said computer readable physiologic pharmacokinetic simulation model comprising differential equations for calculating as a function of time the change in (i) a physiological parameter of one or more of said segments and (ii) a pharmacokinetic property comprising an absorption parameter of a compound relative to a selected route of administration, barrier to absorption and sampling site of one or more of said segments, wherein one or more of said differential equations is modified by a selectively optimized adjustment parameter.

199. An article of manufacture comprising a computer readable medium having embodied therein a computer readable physiologic pharmacokinetic simulation model of at least two segments of a selected mammalian system of interest, said computer readable physiologic pharmacokinetic simulation model comprising as operably linked components:

(i) differential equations for calculating the change in one or more physiological parameters of first and second segments of said mammalian system of interest and the movement and disposition of said compound in said first and second segments as a function of time, and (ii) a logic function module having a regional correlation parameter estimation function and a control statement for initiating said function, wherein said estimation function when initiated is capable of generating an estimated value for a selected pharmacokinetic property comprising an absorption parameter of said compound in said first segment when supplied with an input value corresponding to said selected pharmacokinetic property of said compound in said second segment and with a regional correlation coefficient for said selected pharmacokinetic parameter of said first and second segments.

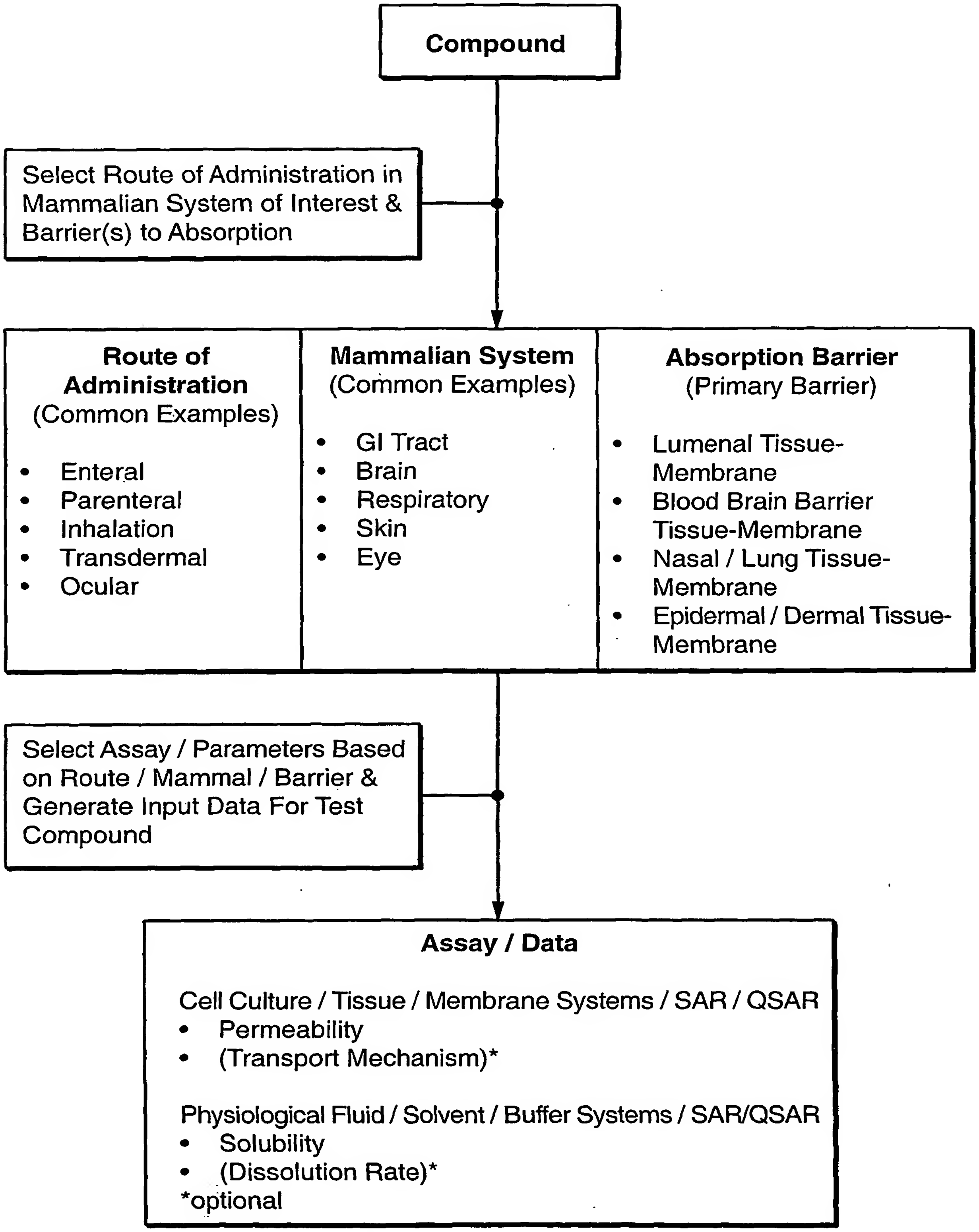

Compound
Select Route of Administration in Mammalian System of Interest & Barrier(s) to Absorption
Route of Administration (Common Examples)
• Enteral
• Parenteral
• Inhalation
• Transdermal
• Ocular
Mammalian System (Common Examples)
• GI Tract
• Brain
• Respiratory
• Skin
• Eye
Absorption Barrier (Primary Barrier)
• Lumenal Tissue-Membrane
• Blood Brain Barrier Tissue-Membrane
• Nasal / Lung Tissue-Membrane
• Epidermal / Dermal Tissue-Membrane
Select Assay / Parameters Based on Route / Mammal / Barrier & Generate Input Data For Test Compound
Assay / Data
Cell Culture / Tissue / Membrane Systems / SAR / QSAR
• Permeability
• (Transport Mechanism)*
Physiological Fluid / Solvent / Buffer Systems / SAR/QSAR
• Solubility
• (Dissolution Rate)*
*optional

FIG._1

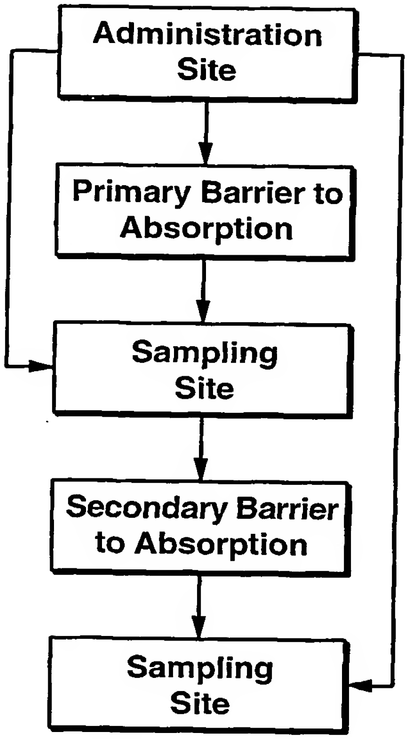

Administration Site
Primary Barrier to Absorption
Sampling Site
Secondary Barrier to Absorption
Sampling Site

FIG._2

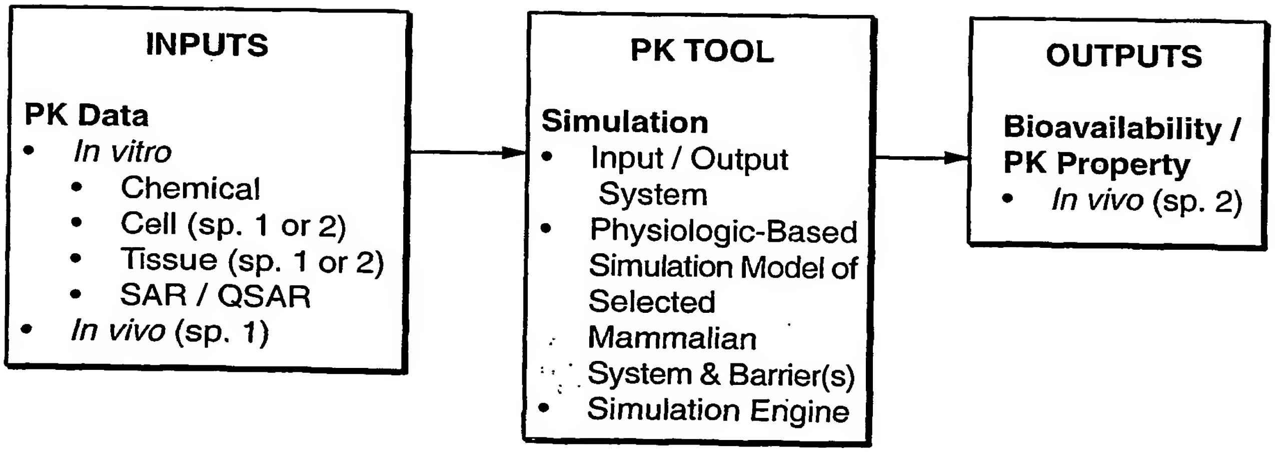

INPUTS
PK Data
• In vitro
• Chemical
• Cell (sp. 1 or 2)
• Tissue (sp. 1 or 2)
• SAR / QSAR
• In vivo (sp. 1)
PK TOOL
Simulation
• Input / Output System
• Physiologic-Based Simulation Model of Selected Mammalian System & Barrier(s)
• Simulation Engine
OUTPUTS
Bioavailability / PK Property
• In vivo (sp. 2)

FIG._3

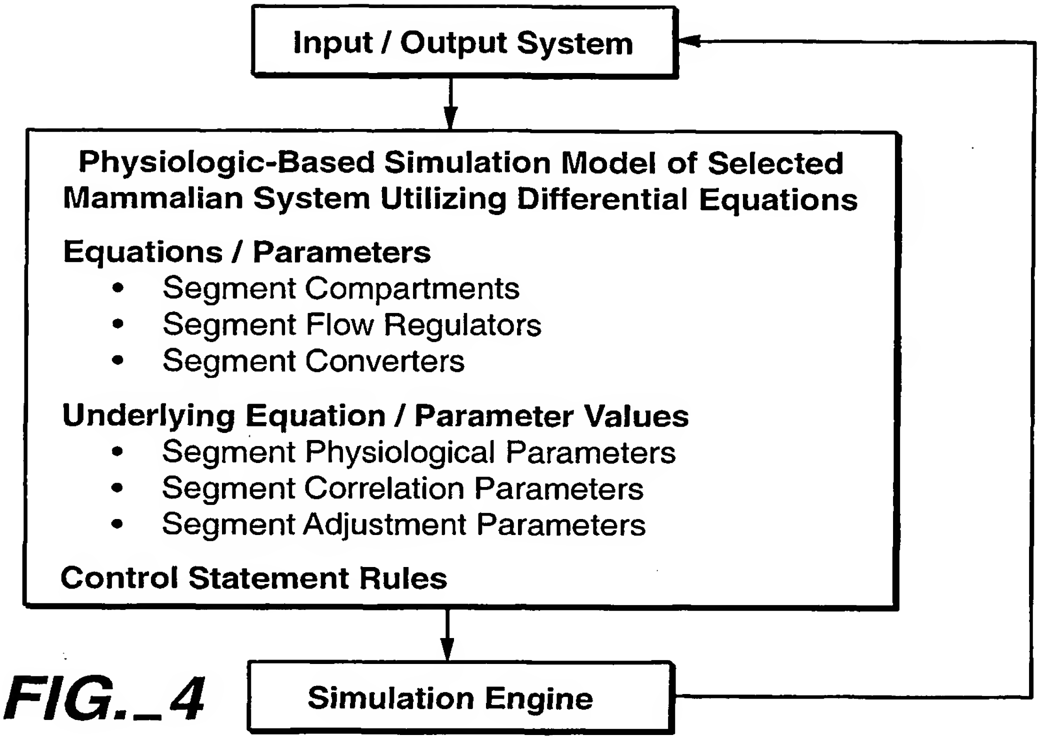

Input / Output System
Physiologic-Based Simulation Model of Selected Mammalian System Utilizing Differential Equations
Equations / Parameters
• Segment Compartments
• Segment Flow Regulators
• Segment Converters
Underlying Equation / Parameter Values
• Segment Physiological Parameters
• Segment Correlation Parameters
• Segment Adjustment Parameters
Control Statement Rules
Simulation Engine

FIG._4

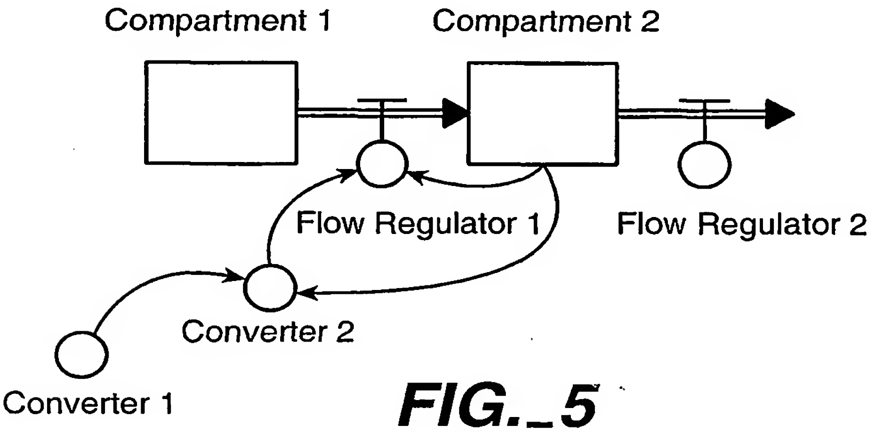

Compartment 1
Compartment 2
Flow Regulator 1
Flow Regulator 2
Converter 2
Converter 1

FIG._5

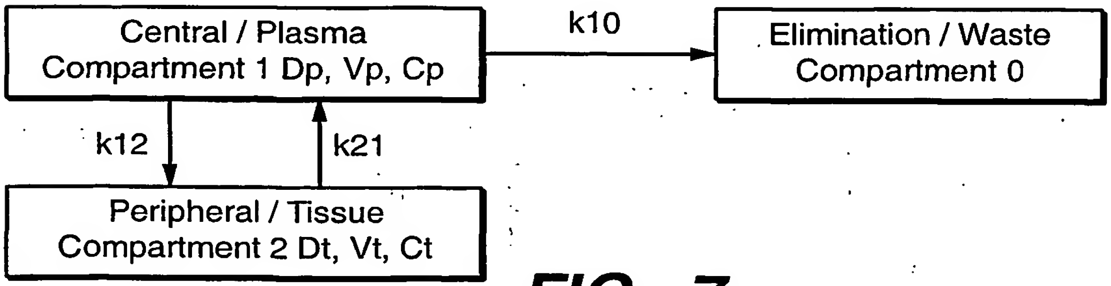

Central / Plasma Compartment 1 Dp, Vp, Cp
k10
Elimination / Waste Compartment 0
k12
k21
Peripheral / Tissue Compartment 2 Dt, Vt, Ct

FIG._7

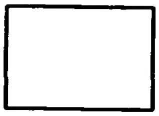
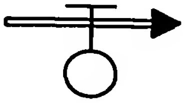
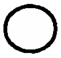
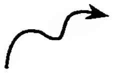
Symbol	**Name**	**Time-Dependent Function**
	Compartment	Equation or value for amount of substance stored.
	Flow Regulator	Rate equation for amount of substance transferred.
	Converter	Equation or pre-defined value for (i) input into flow regulator; (ii) input into another converter; and / or (iii) storing value.
	Input Link	Directs input values.

FIG._6

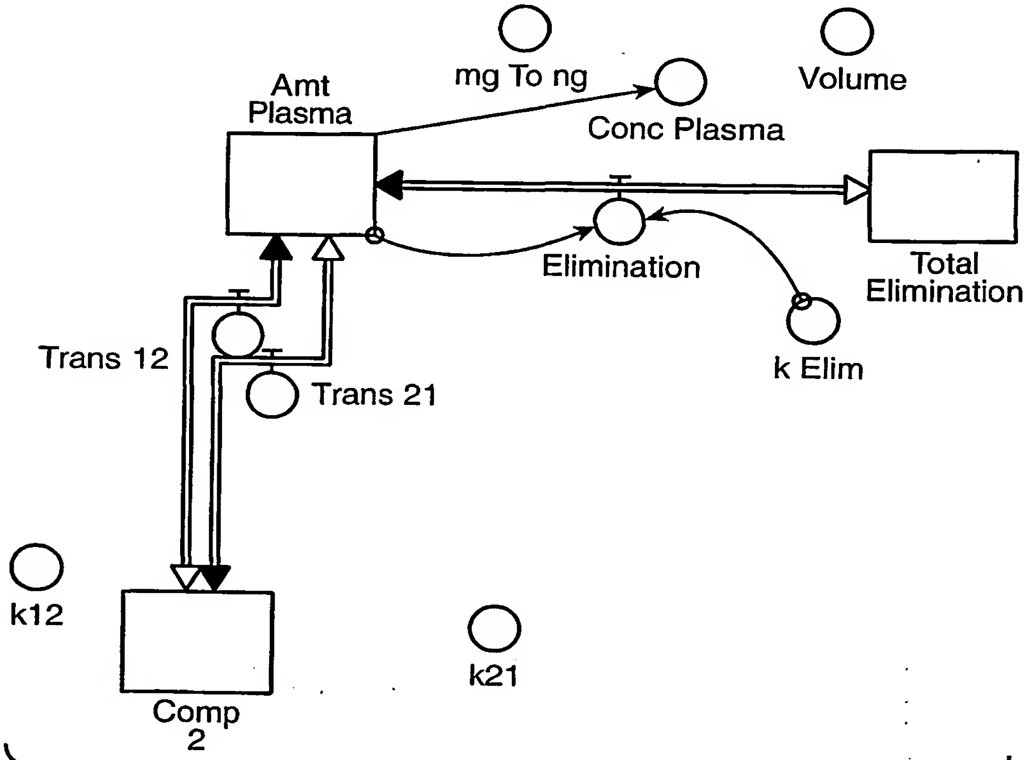

Amt Plasma
mg To ng
Conc Plasma
Volume
Total Elimination
Elimination
k Elim
Trans 12
Trans 21
k12
k21
Comp 2

FIG._8

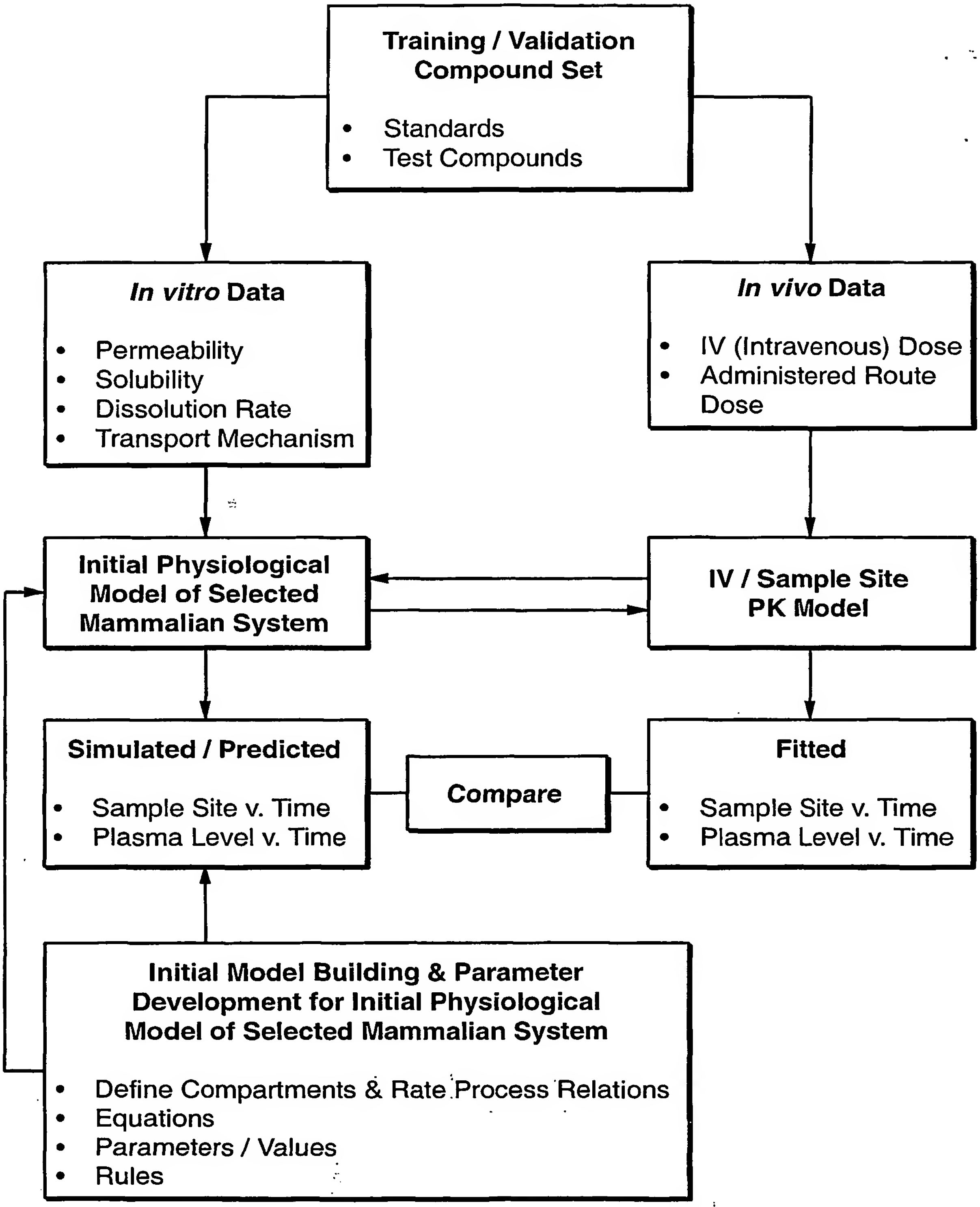

Training / Validation Compound Set
• Standards
• Test Compounds
In vitro Data
• Permeability
• Solubility
• Dissolution Rate
• Transport Mechanism
In vivo Data
• IV (Intravenous) Dose
• Administered Route Dose
Initial Physiological Model of Selected Mammalian System
IV / Sample Site PK Model
Simulated / Predicted
• Sample Site v. Time
• Plasma Level v. Time
Compare
Fitted
• Sample Site v. Time
• Plasma Level v. Time
Initial Model Building & Parameter Development for Initial Physiological Model of Selected Mammalian System
• Define Compartments & Rate Process Relations
• Equations
• Parameters / Values
• Rules

FIG._9

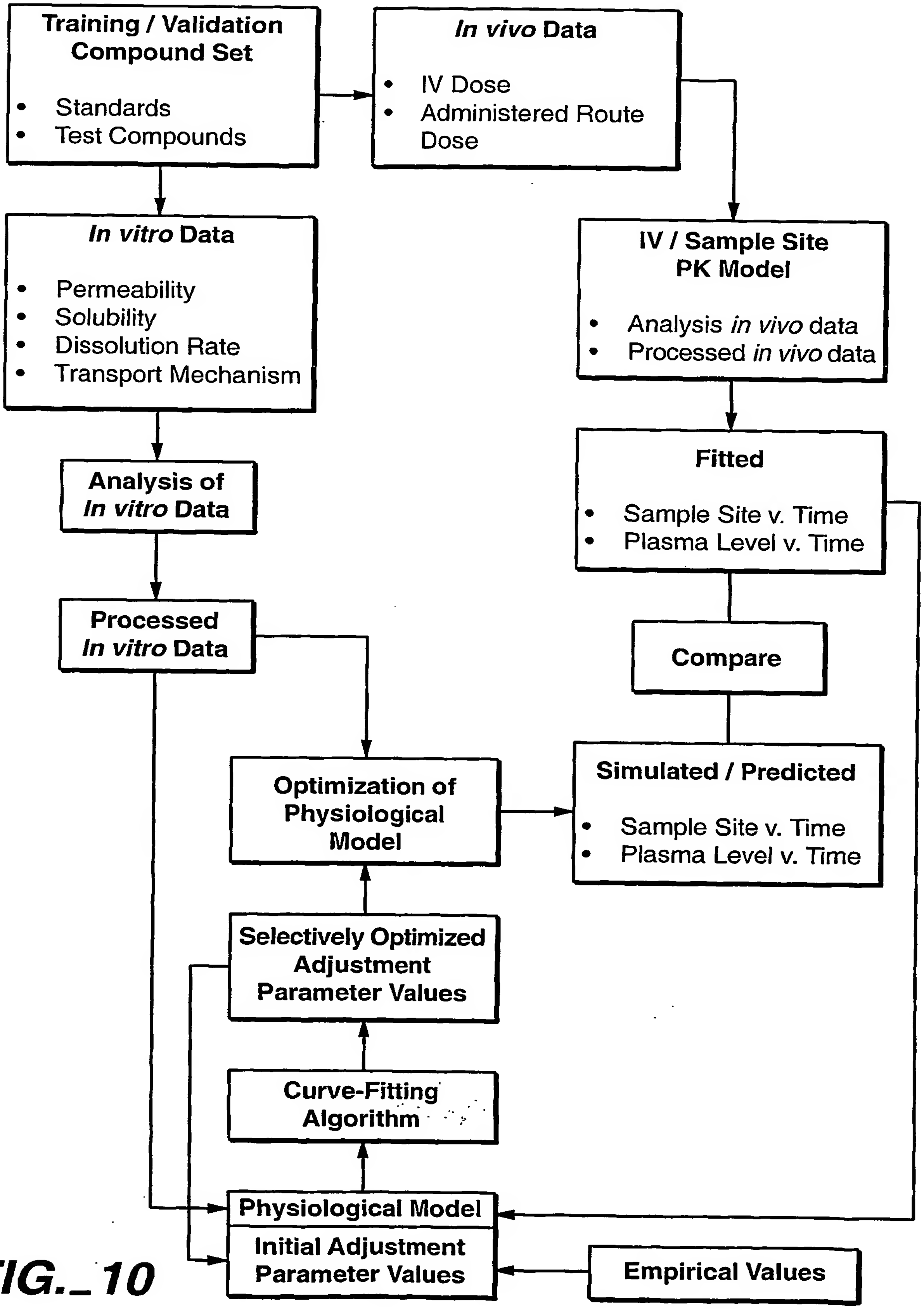

Training / Validation Compound Set
• Standards
• Test Compounds
In vivo Data
• IV Dose
• Administered Route Dose
In vitro Data
• Permeability
• Solubility
• Dissolution Rate
• Transport Mechanism
IV / Sample Site PK Model
• Analysis in vivo data
• Processed in vivo data
Analysis of In vitro Data
Fitted
• Sample Site v. Time
• Plasma Level v. Time
Processed In vitro Data
Compare
Optimization of Physiological Model
Simulated / Predicted
• Sample Site v. Time
• Plasma Level v. Time
Selectively Optimized Adjustment Parameter Values
Curve-Fitting Algorithm
Physiological Model
Initial Adjustment Parameter Values
Empirical Values

FIG._10

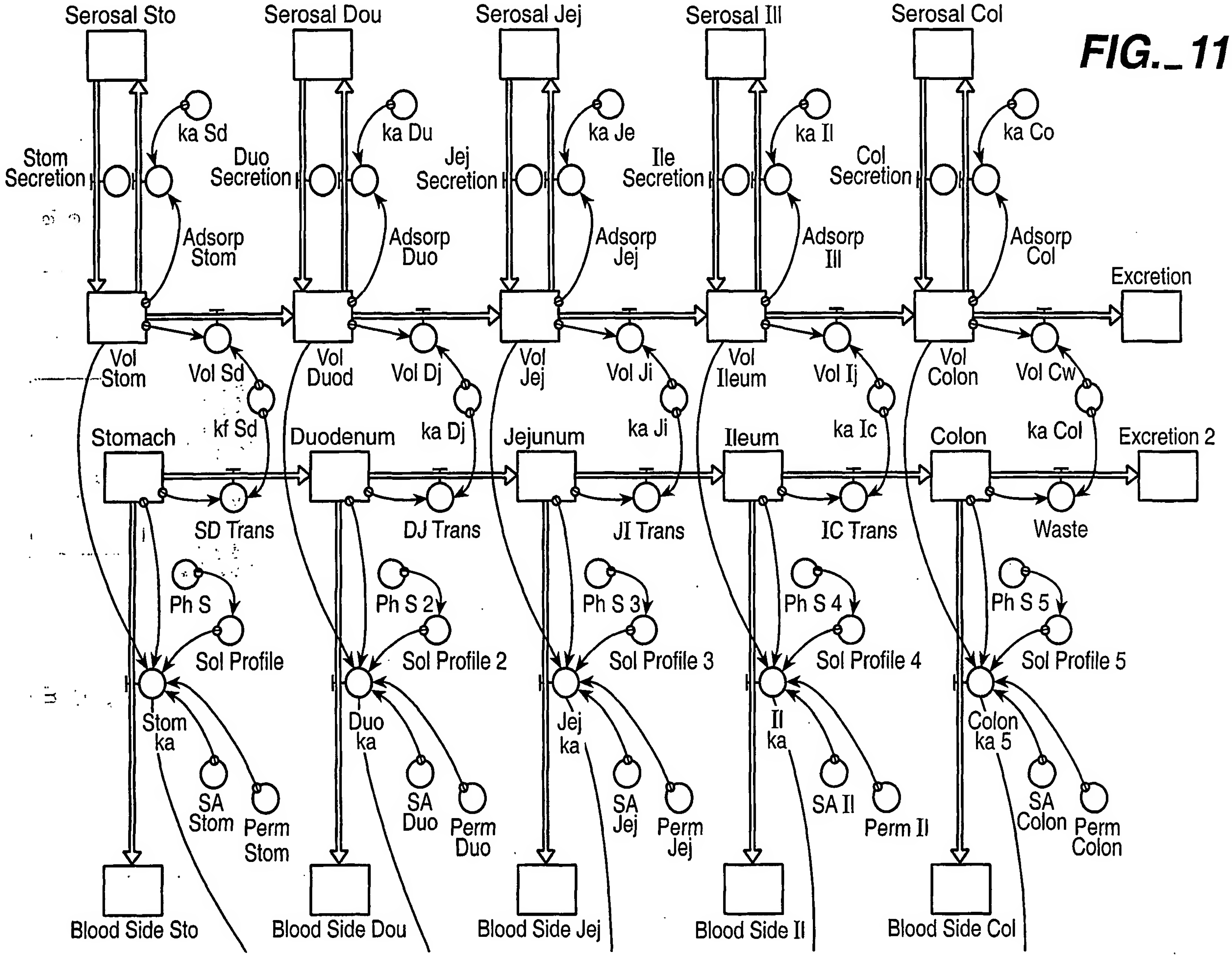

Serosal Sto
Serosal Dou
Serosal Jej
Serosal Ill
Serosal Col
ka Sd
ka Du
ka Je
ka Il
ka Co
Stom Secretion
Duo Secretion
Jej Secretion
Ile Secretion
Col Secretion
Adsorp Stom
Adsorp Duo
Adsorp Jej
Adsorp Ill
Adsorp Col
Excretion
Vol Stom
Vol Sd
Vol Duod
Vol Dj
Vol Jej
Vol Ji
Vol Ileum
Vol Ij
Vol Colon
Vol Cw
kf Sd
ka Dj
ka Ji
ka Ic
ka Col
Stomach
Duodenum
Jejunum
Ileum
Colon
Excretion 2
SD Trans
DJ Trans
JI Trans
IC Trans
Waste
Ph S
Ph S 2
Ph S 3
Ph S 4
Ph S 5
Sol Profile
Sol Profile 2
Sol Profile 3
Sol Profile 4
Sol Profile 5
Stom ka
Duo ka
Jej ka
Il ka
Colon ka 5
SA Stom
SA Duo
SA Jej
SA Il
SA Colon
Perm Stom
Perm Duo
Perm Jej
Perm Il
Perm Colon
Blood Side Sto
Blood Side Dou
Blood Side Jej
Blood Side Il
Blood Side Col

FIG._11-A

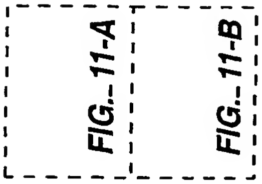


FIG._11-A
FIG._11-B

FIG._11

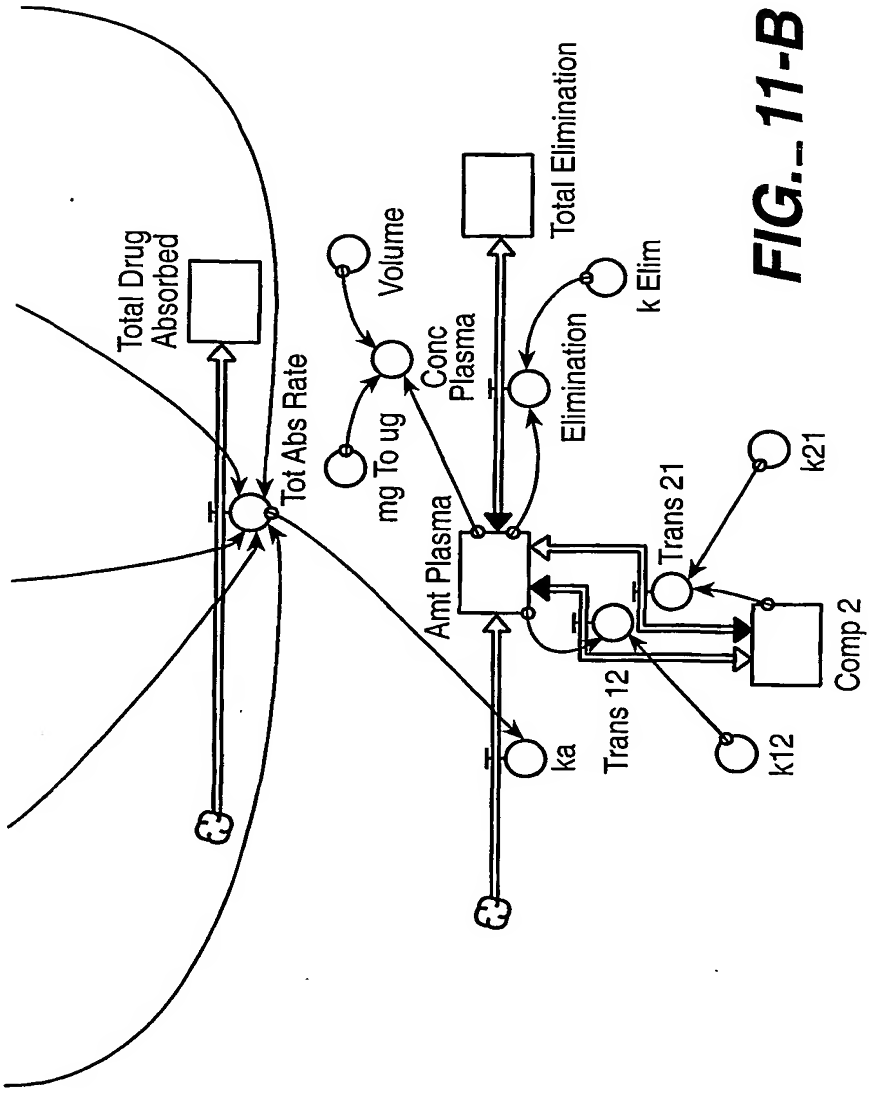

Total Drug Absorbed
Tot Abs Rate
Volume
Conc Plasma
mg To ug
Total Elimination
Elimination
k Elim
Amt Plasma
Trans 21
k21
Comp 2
Trans 12
k12
ka

FIG._11-B

Mass-Volume GI Tract Model

- **GI Segment Compartments**
  - Fluid Volume
  - Fluid Absorption
  - Insoluble Mass
  - Soluble Mass Absorption
- **GI Segment Flow Regulators**
  - Fluid Volume Absorption Rate
  - Fluid Volume Secretion Rate
  - Fluid Volume GI Transit Rate
  - Insoluble Mass GI Transit Rate
  - Soluble Mass Absorption Rate
- **GI Segment Converters**
  - Rate Constant
  - pH
  - Solubility
  - Surface Area
  - Permeability

FIG._12

Mass-Volume GI Tract Model

- **GI Segment Compartments & *Flow Regulators***
  - **Fluid Volume**
    - *Fluid Volume Absorption Rate*
    - *Fluid Volume Secretion Rate*
    - *Fluid Volume GI Transit Rate*
  - **Fluid Volume Absorption**
    - *Fluid Volume Absorption Rate*
    - *Fluid Volume Secretion Rate*
  - **Insoluble Mass**
    - *Insoluble Mass GI Transit Rate*
    - *Soluble Mass Absorption Rate*
  - **Soluble Mass Absorption**
    - *Soluble Mass Absorption Rate*

FIG._13

Mass-Volume GI Tract Model

- **GI Segment Flow Regulators & *Converters***
  - **Fluid Volume Absorption Rate**
    - *Fluid Volume Absorption Rate Constant*
  - **Fluid Volume Secretion Rate**
    - *Fluid Volume Secretion Rate Constant*
  - **Fluid Volume GI Transit Rate**
    - *Fluid Volume GI Transit Rate Constant*
  - **Insoluble Mass GI Transit Rate**
    - *Insoluble Mass GI Transit Rate Constant*
  - **Soluble Mass Absorption Rate**
    - *Fluid Volume*
    - *Insoluble Mass*
    - *Mass Solubility Profile*
      - *pH*
    - *Permeability*
    - *Surface Area*

FIG._14

Mass-Volume GI Tract Model

- **GI Segment Converters**
  - Rate Constant
  - pH
  - Solubility
  - Surface Area
  - Permeability

FIG._15

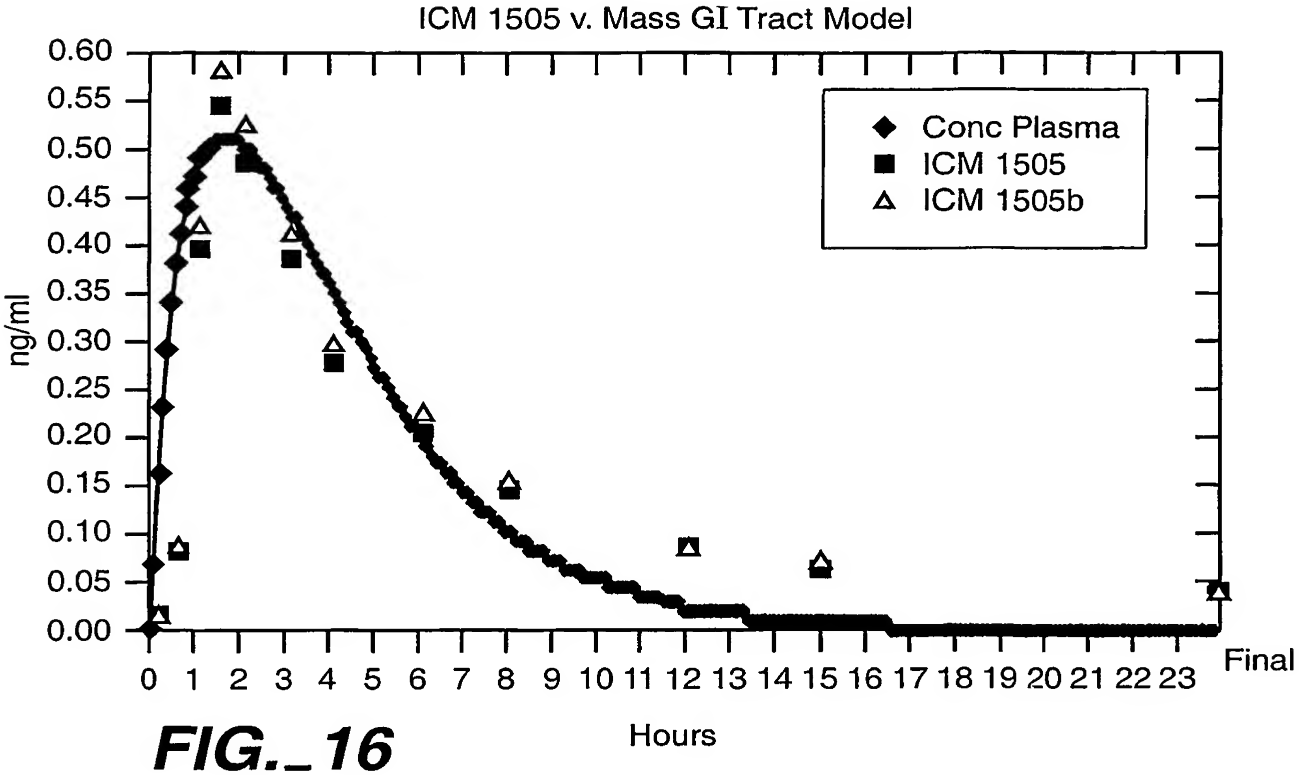

ICM 1505 v. Mass GI Tract Model
Conc Plasma
ICM 1505
ICM 1505b
ng/ml
0.60
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
Final
Hours

FIG._16

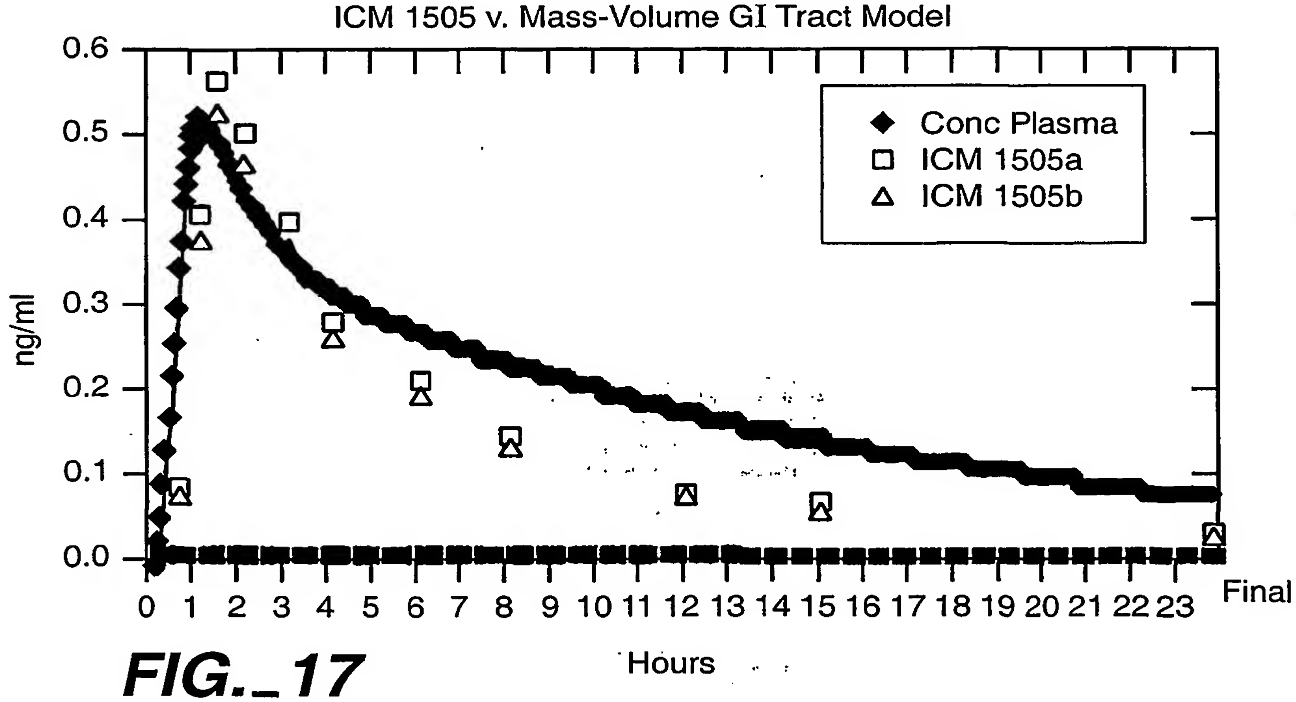

ICM 1505 v. Mass-Volume GI Tract Model
Conc Plasma
ICM 1505a
ICM 1505b
ng/ml
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
Final
Hours

FIG._17

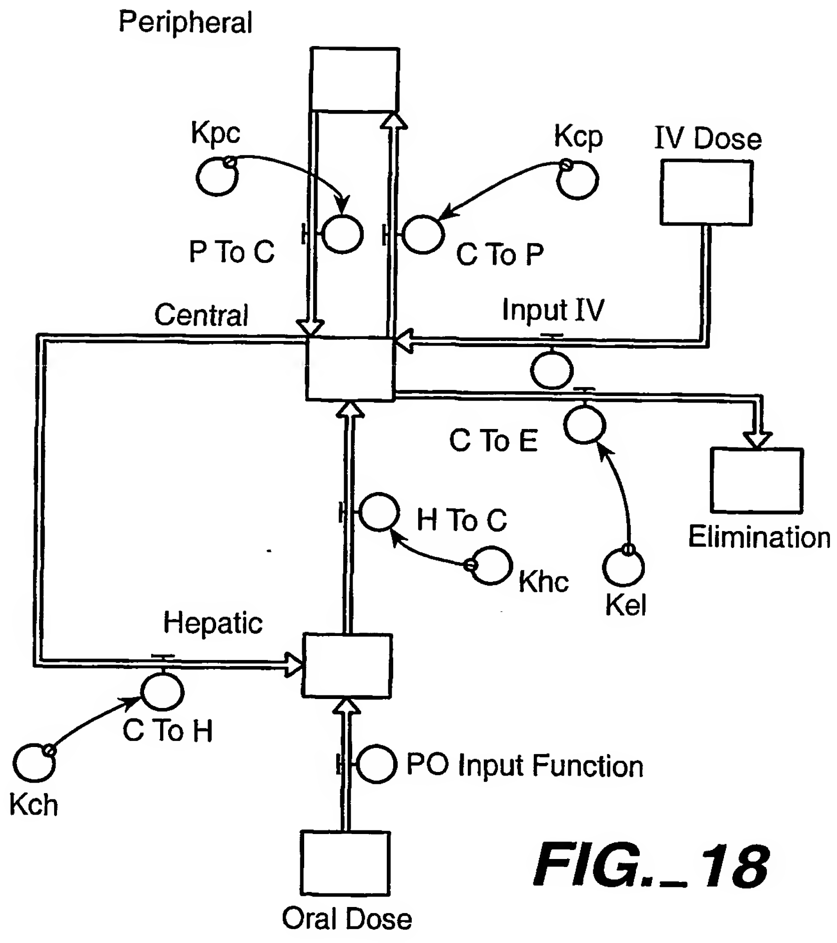

Peripheral
Kpc
Kcp
IV Dose
P To C
C To P
Central
Input IV
C To E
Elimination
H To C
Khc
Kel
Hepatic
C To H
Kch
PO Input Function
Oral Dose

FIG._18

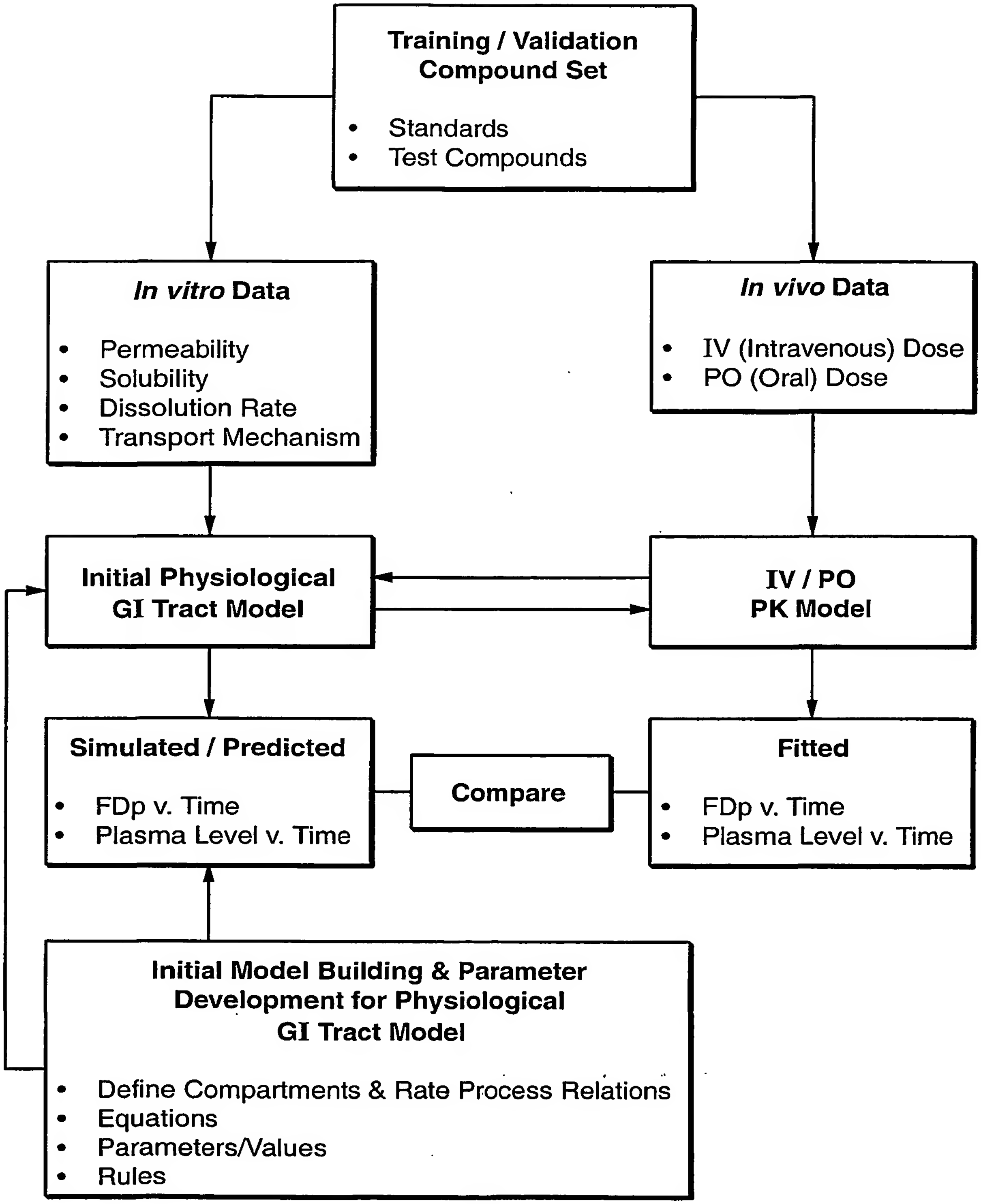

Training / Validation Compound Set
• Standards
• Test Compounds
In vitro Data
• Permeability
• Solubility
• Dissolution Rate
• Transport Mechanism
In vivo Data
• IV (Intravenous) Dose
• PO (Oral) Dose
Initial Physiological GI Tract Model
IV / PO PK Model
Simulated / Predicted
• FDp v. Time
• Plasma Level v. Time
Compare
Fitted
• FDp v. Time
• Plasma Level v. Time
Initial Model Building & Parameter Development for Physiological GI Tract Model
• Define Compartments & Rate Process Relations
• Equations
• Parameters/Values
• Rules

FIG._19

Gastrointestinal Transit

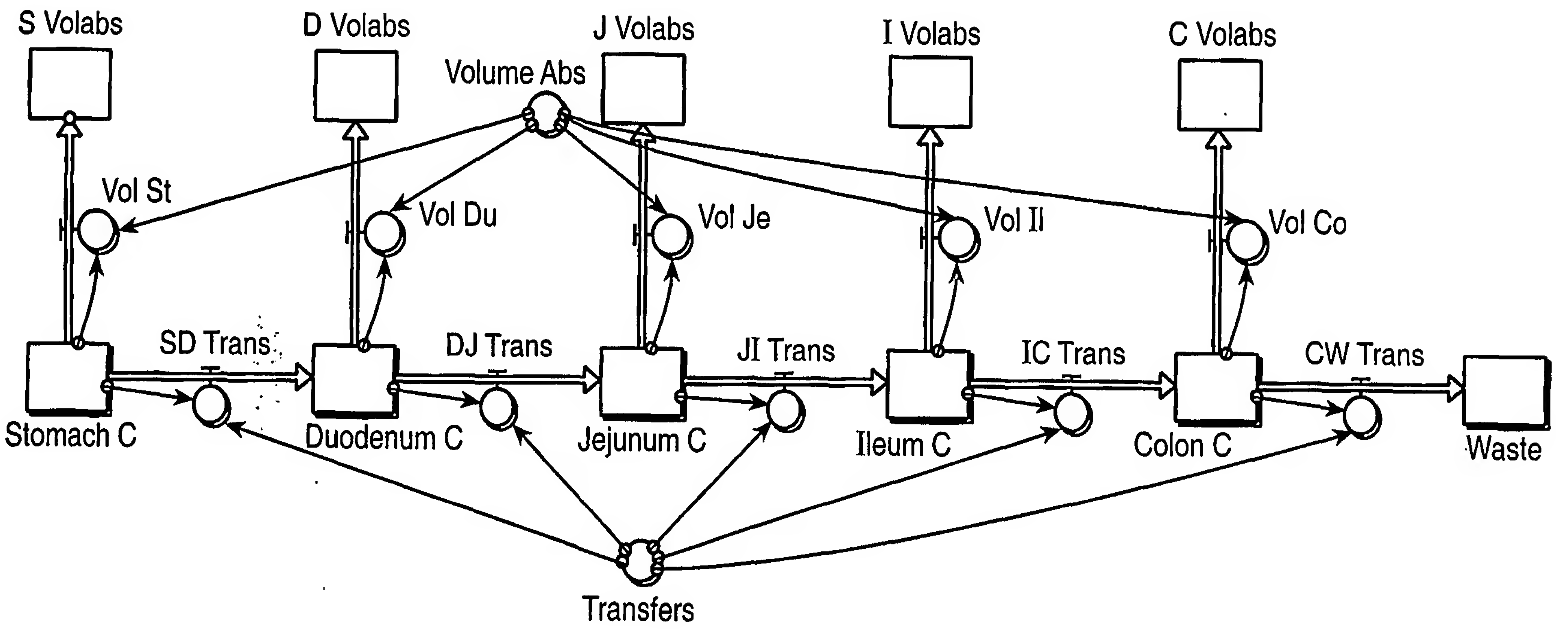

S Volabs
D Volabs
J Volabs
I Volabs
C Volabs
Volume Abs
Vol St
Vol Du
Vol Je
Vol Il
Vol Co
SD Trans
DJ Trans
JI Trans
IC Trans
CW Trans
Stomach C
Duodenum C
Jejunum C
Ileum C
Colon C
Waste
Transfers

FIG._20

pH Dependent Solubility And Dissolution

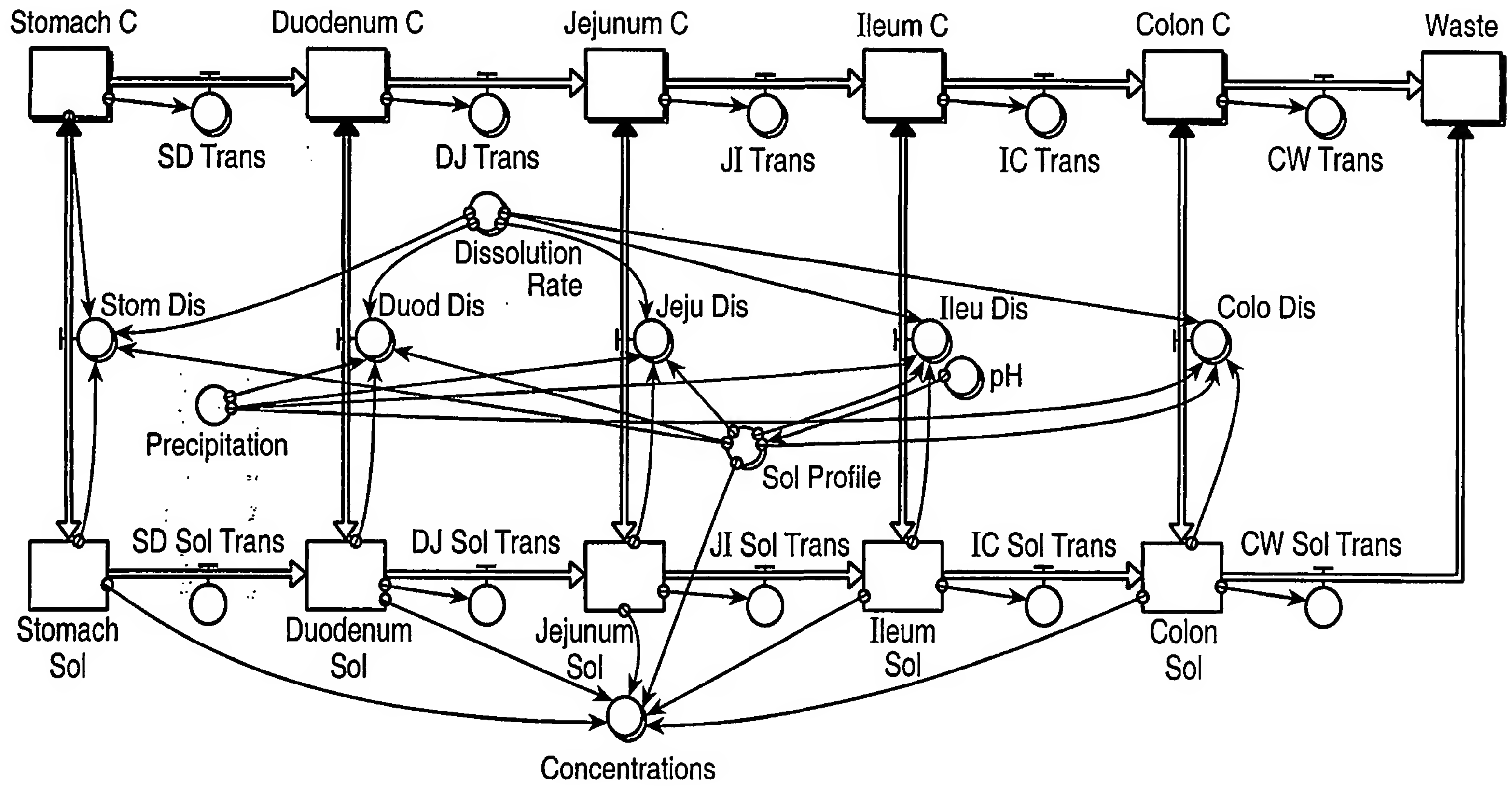

Stomach C
Duodenum C
Jejunum C
Ileum C
Colon C
Waste
SD Trans
DJ Trans
JI Trans
IC Trans
CW Trans
Dissolution Rate
Stom Dis
Duod Dis
Jeju Dis
Ileu Dis
Colo Dis
pH
Precipitation
Sol Profile
SD Sol Trans
DJ Sol Trans
JI Sol Trans
IC Sol Trans
CW Sol Trans
Stomach Sol
Duodenum Sol
Jejunum Sol
Ileum Sol
Colon Sol
Concentrations

FIG._21

Absorption

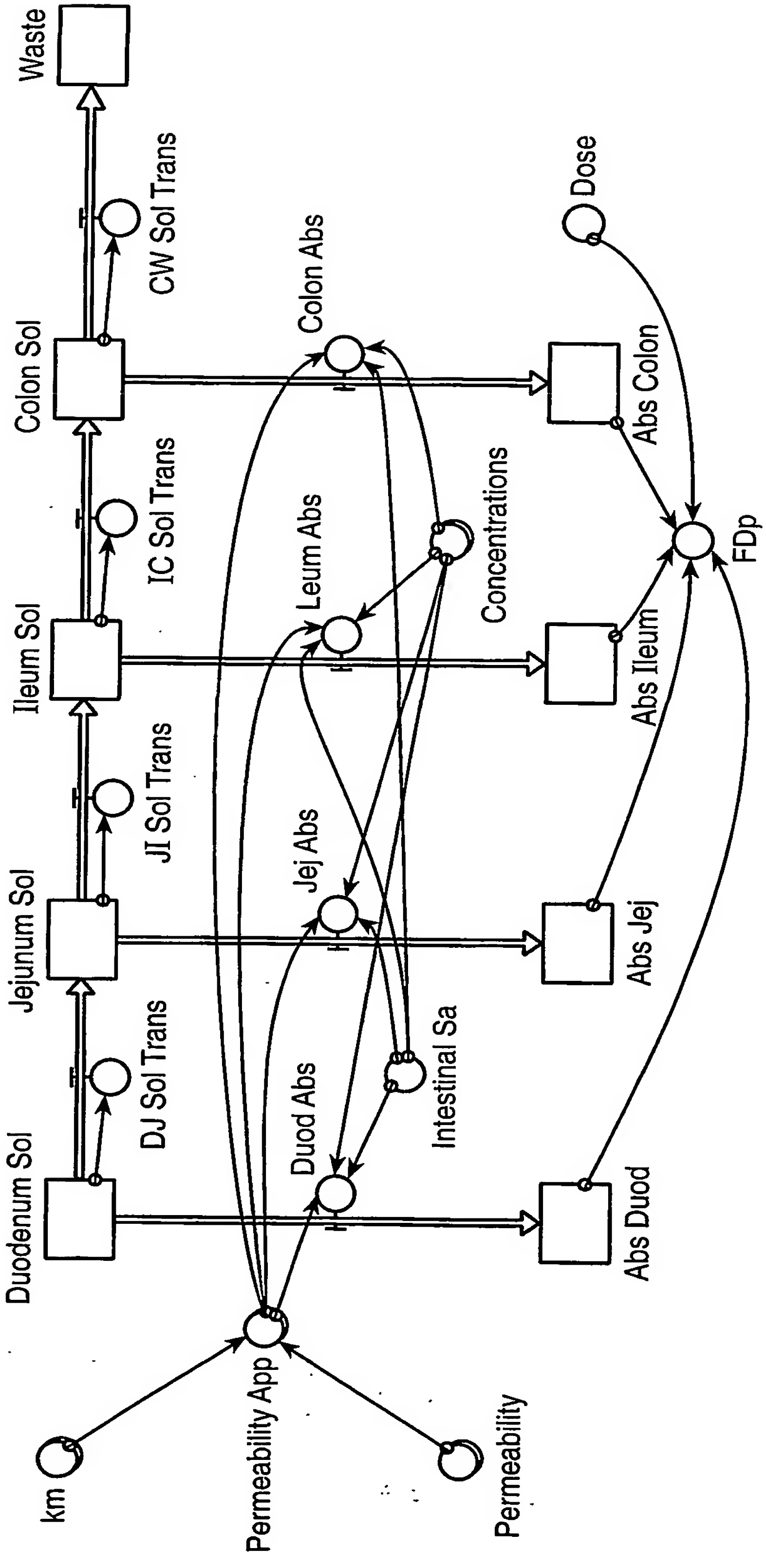

Waste
CW Sol Trans
Colon Sol
IC Sol Trans
Ileum Sol
JI Sol Trans
Jejunum Sol
DJ Sol Trans
Duodenum Sol
Colon Abs
Leum Abs
Jej Abs
Duod Abs
Concentrations
Intestinal Sa
Dose
Abs Colon
Abs Ileum
Abs Jej
Abs Duod
FDp
km
Permeability App
Permeability

FIG._22

GI Tract - Intestinal Model

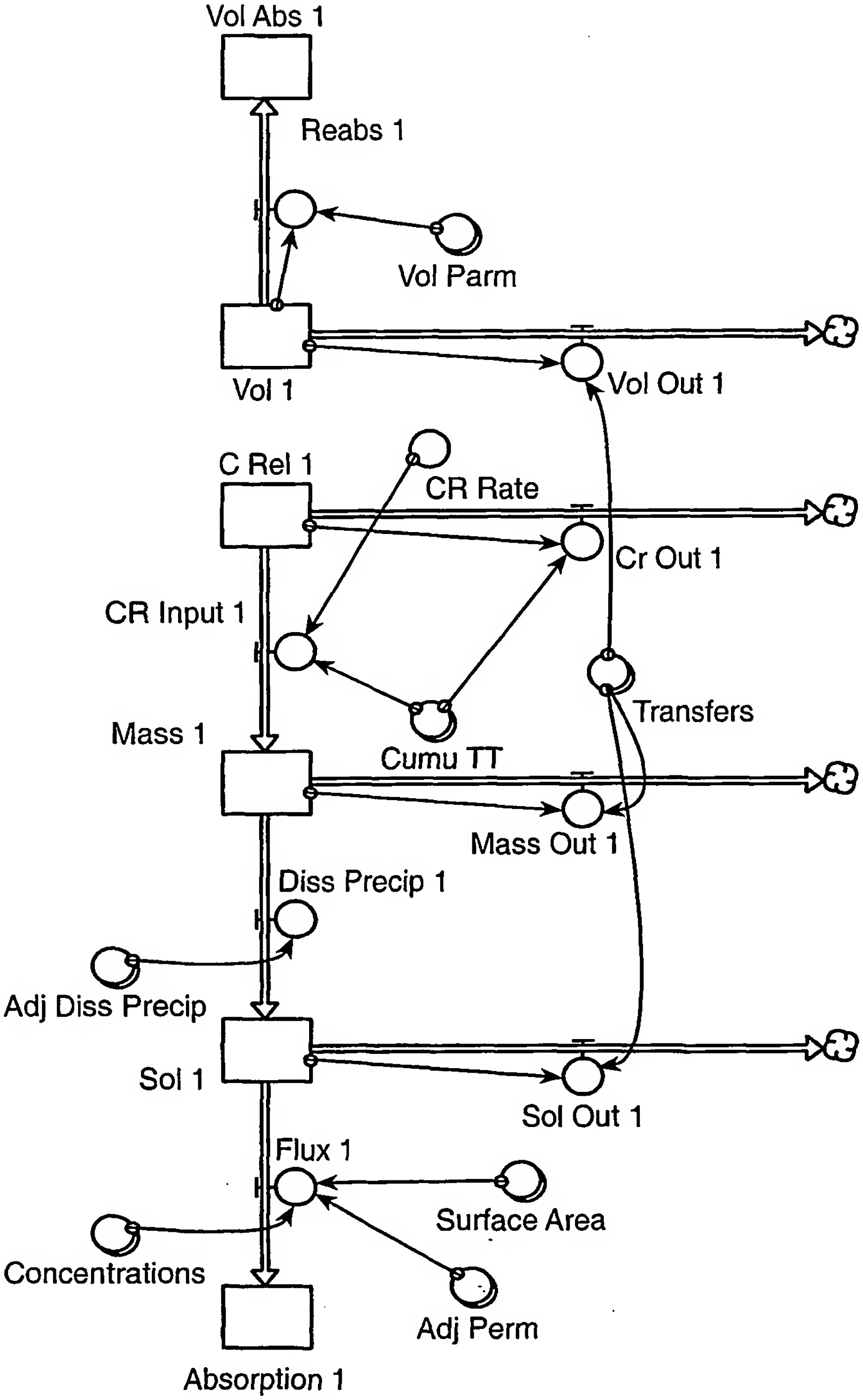

Vol Abs 1
Reabs 1
Vol Parm
Vol 1
Vol Out 1
C Rel 1
CR Rate
Cr Out 1
CR Input 1
Transfers
Cumu TT
Mass 1
Mass Out 1
Diss Precip 1
Adj Diss Precip
Sol 1
Sol Out 1
Flux 1
Surface Area
Concentrations
Adj Perm
Absorption 1

FIG._23

FIG._24-A

GI Tract-Intestinal Model (Without Converters, Ghosts or Connectors)

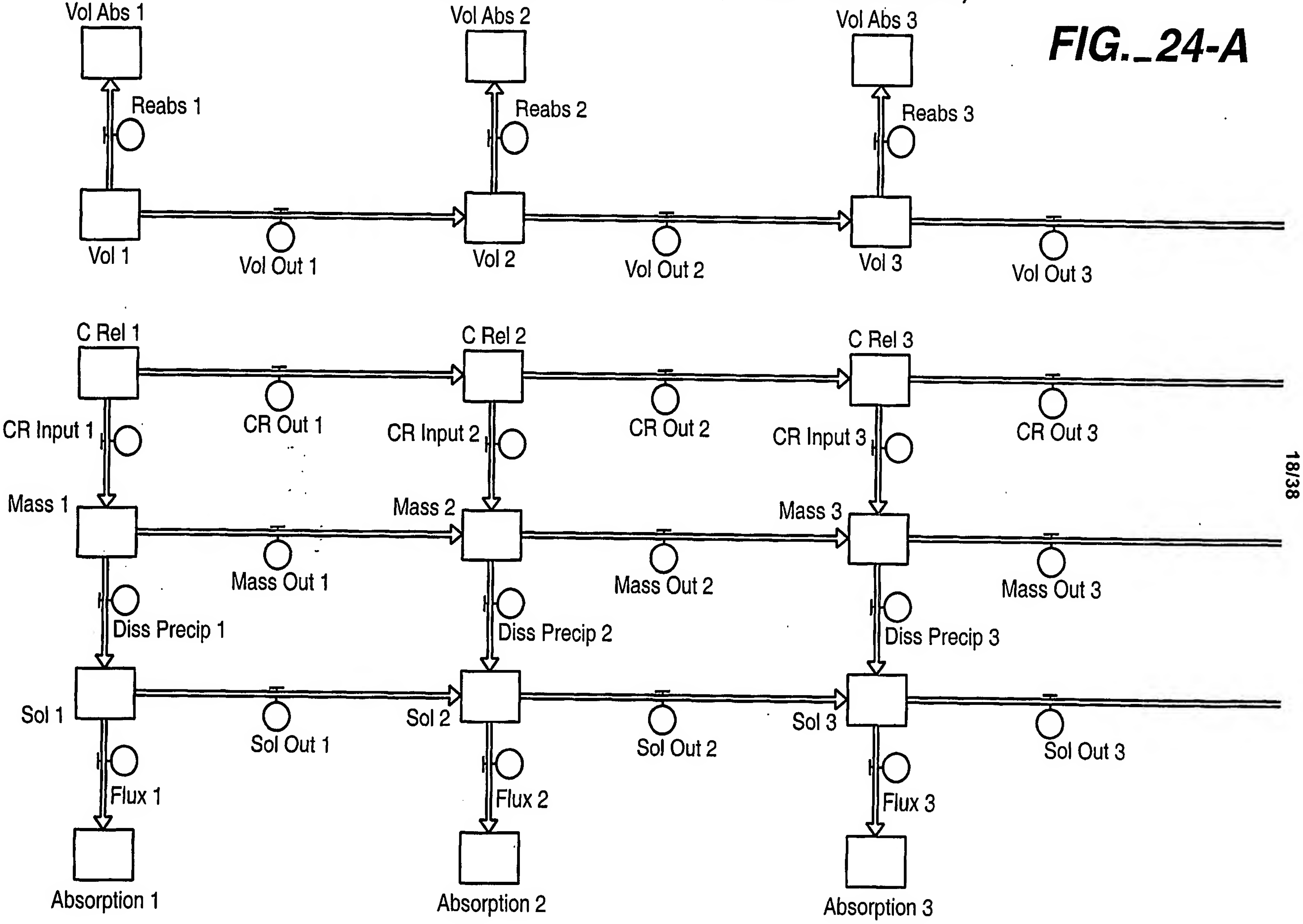
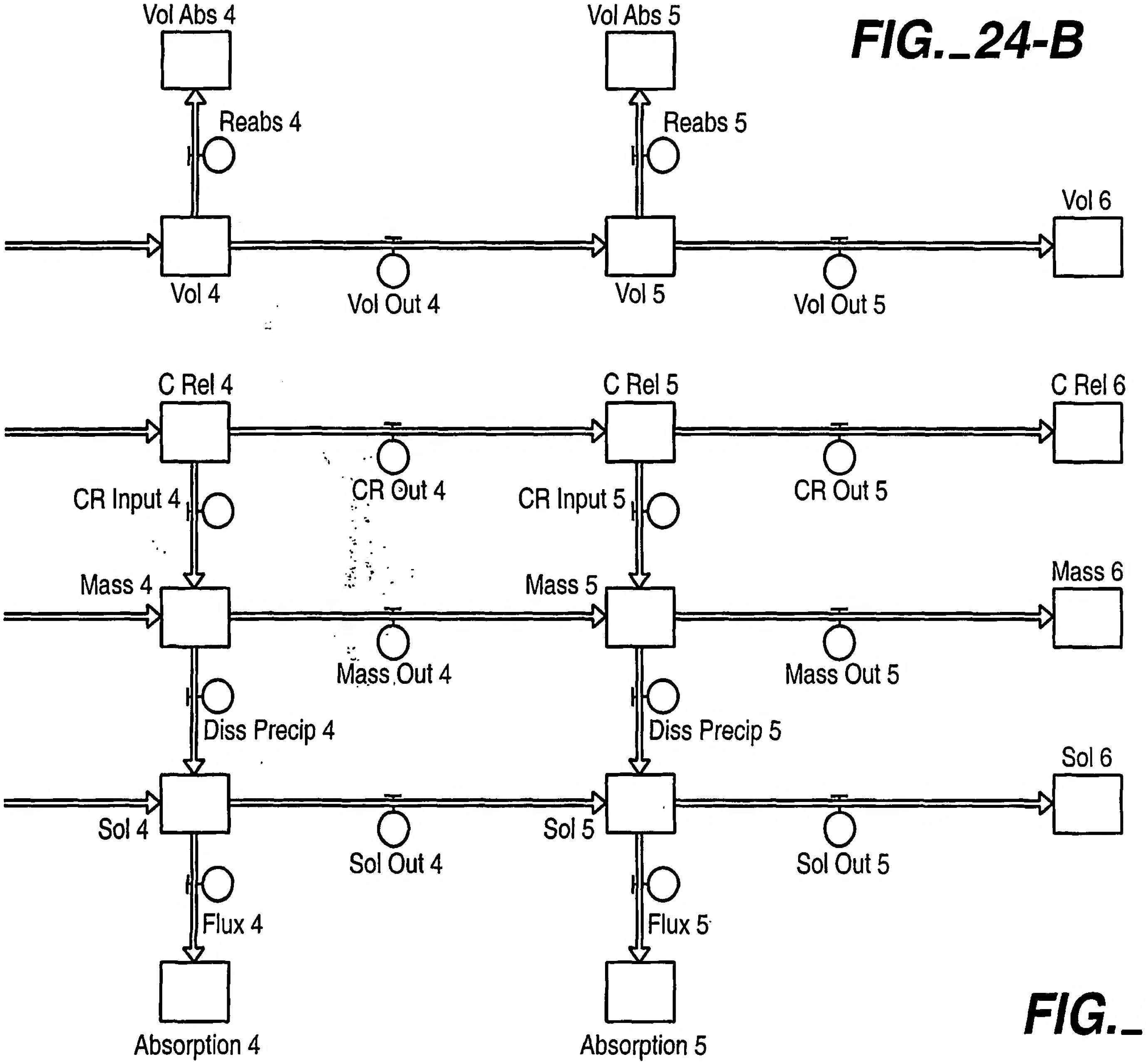

Vol Abs 1
Vol Abs 2
Vol Abs 3
Reabs 1
Reabs 2
Reabs 3
Vol 1
Vol Out 1
Vol 2
Vol Out 2
Vol 3
Vol Out 3
C Rel 1
C Rel 2
C Rel 3
CR Input 1
CR Out 1
CR Input 2
CR Out 2
CR Input 3
CR Out 3
Mass 1
Mass 2
Mass 3
Mass Out 1
Mass Out 2
Mass Out 3
Diss Precip 1
Diss Precip 2
Diss Precip 3
Sol 1
Sol Out 1
Sol 2
Sol Out 2
Sol 3
Sol Out 3
Flux 1
Flux 2
Flux 3
Absorption 1
Absorption 2
Absorption 3

FIG._24-B


Vol Abs 4
Vol Abs 5
Reabs 4
Reabs 5
Vol 6
Vol 4
Vol Out 4
Vol 5
Vol Out 5
C Rel 4
C Rel 5
C Rel 6
CR Input 4
CR Out 4
CR Input 5
CR Out 5
Mass 4
Mass 5
Mass 6
Mass Out 4
Mass Out 5
Diss Precip 4
Diss Precip 5
Sol 6
Sol 4
Sol 5
Sol Out 4
Sol Out 5
Flux 4
Flux 5
Absorption 4
Absorption 5

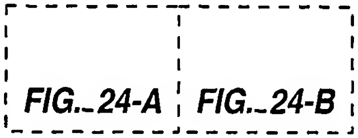


FIG._24-A
FIG._24-B

FIG._24

FIG._25-A

GI Tract-Intestinal Model

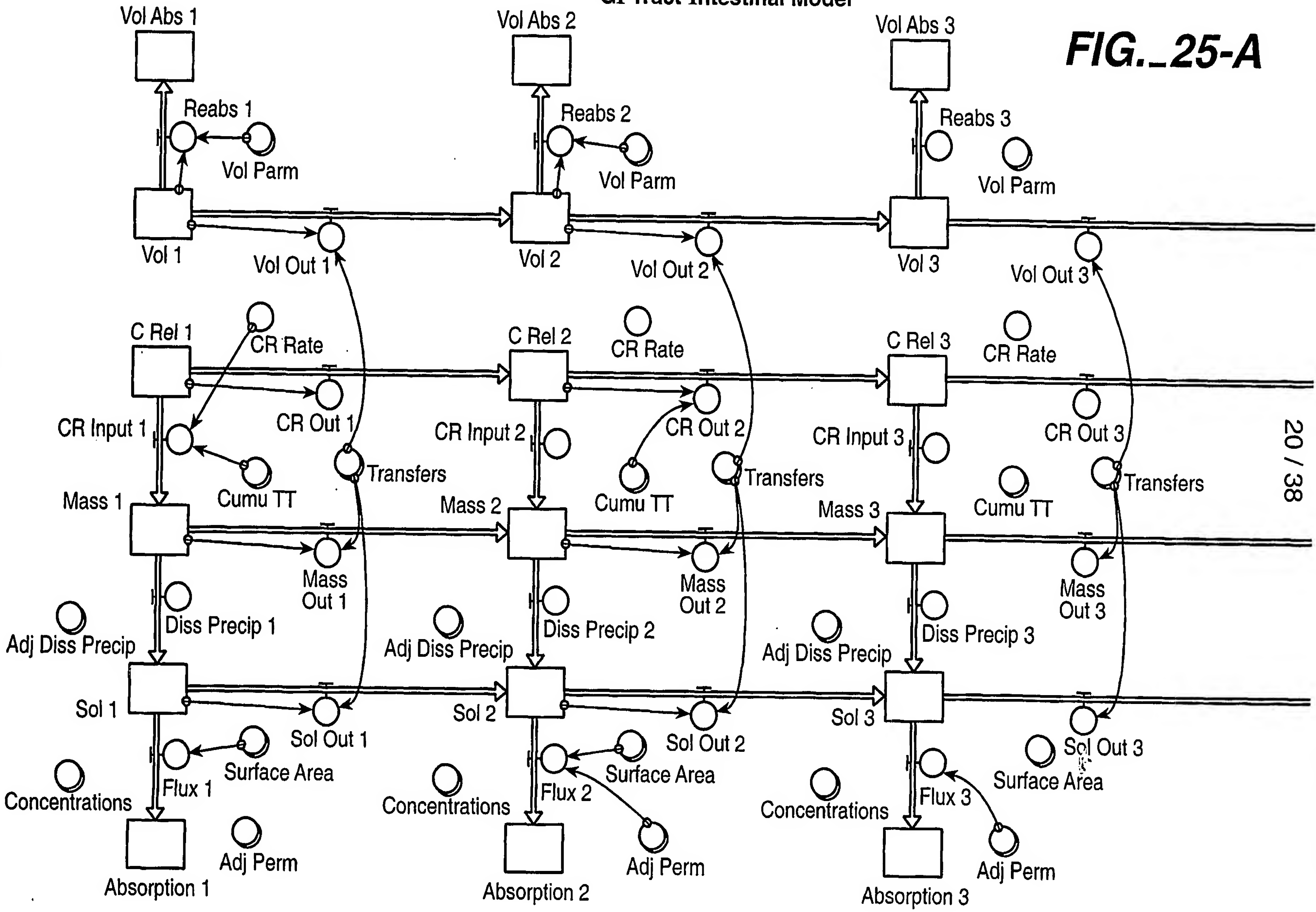
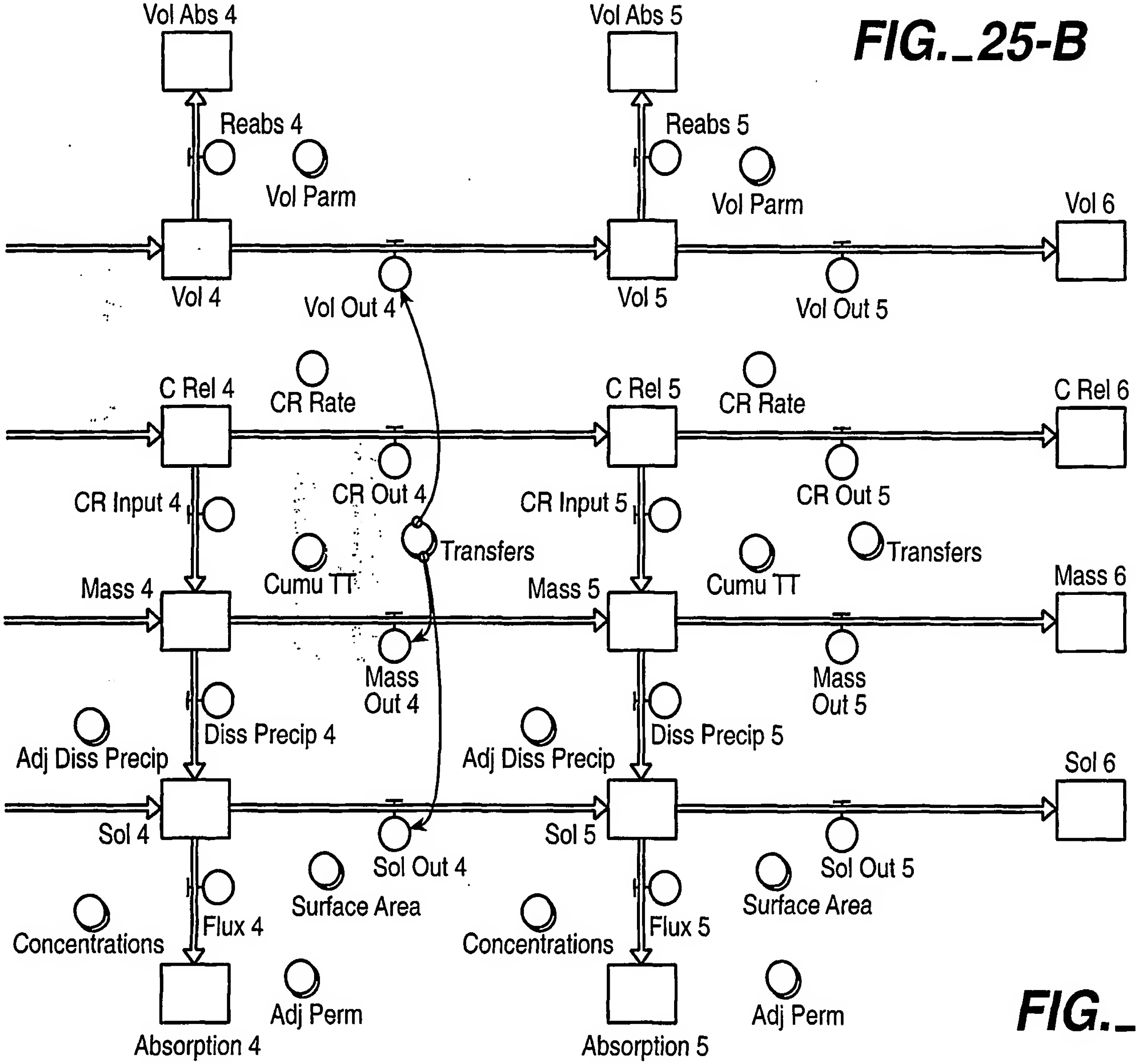

Vol Abs 1
Reabs 1
Vol Parm
Vol 1
Vol Out 1
Vol Abs 2
Reabs 2
Vol Parm
Vol 2
Vol Out 2
Vol Abs 3
Reabs 3
Vol Parm
Vol 3
Vol Out 3
C Rel 1
CR Rate
CR Input 1
CR Out 1
Cumu TT
Transfers
C Rel 2
CR Rate
CR Input 2
CR Out 2
Cumu TT
Transfers
C Rel 3
CR Rate
CR Input 3
CR Out 3
Cumu TT
Transfers
Mass 1
Mass Out 1
Diss Precip 1
Adj Diss Precip
Mass 2
Mass Out 2
Diss Precip 2
Adj Diss Precip
Mass 3
Mass Out 3
Diss Precip 3
Adj Diss Precip
Sol 1
Sol Out 1
Surface Area
Flux 1
Concentrations
Adj Perm
Absorption 1
Sol 2
Sol Out 2
Surface Area
Flux 2
Concentrations
Adj Perm
Absorption 2
Sol 3
Sol Out 3
Surface Area
Flux 3
Concentrations
Adj Perm
Absorption 3

FIG._25-B


Vol Abs 4
Reabs 4
Vol Parm
Vol 4
Vol Out 4
Vol Abs 5
Reabs 5
Vol Parm
Vol 5
Vol Out 5
Vol 6
C Rel 4
CR Rate
CR Out 4
CR Input 4
Transfers
Cumu TT
C Rel 5
CR Rate
CR Out 5
CR Input 5
Transfers
Cumu TT
C Rel 6
Mass 4
Mass Out 4
Diss Precip 4
Adj Diss Precip
Mass 5
Mass Out 5
Diss Precip 5
Adj Diss Precip
Mass 6
Sol 4
Sol Out 4
Surface Area
Flux 4
Concentrations
Adj Perm
Absorption 4
Sol 5
Sol Out 5
Surface Area
Flux 5
Concentrations
Adj Perm
Absorption 5
Sol 6

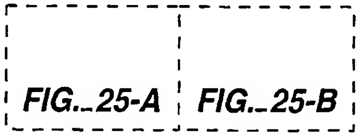
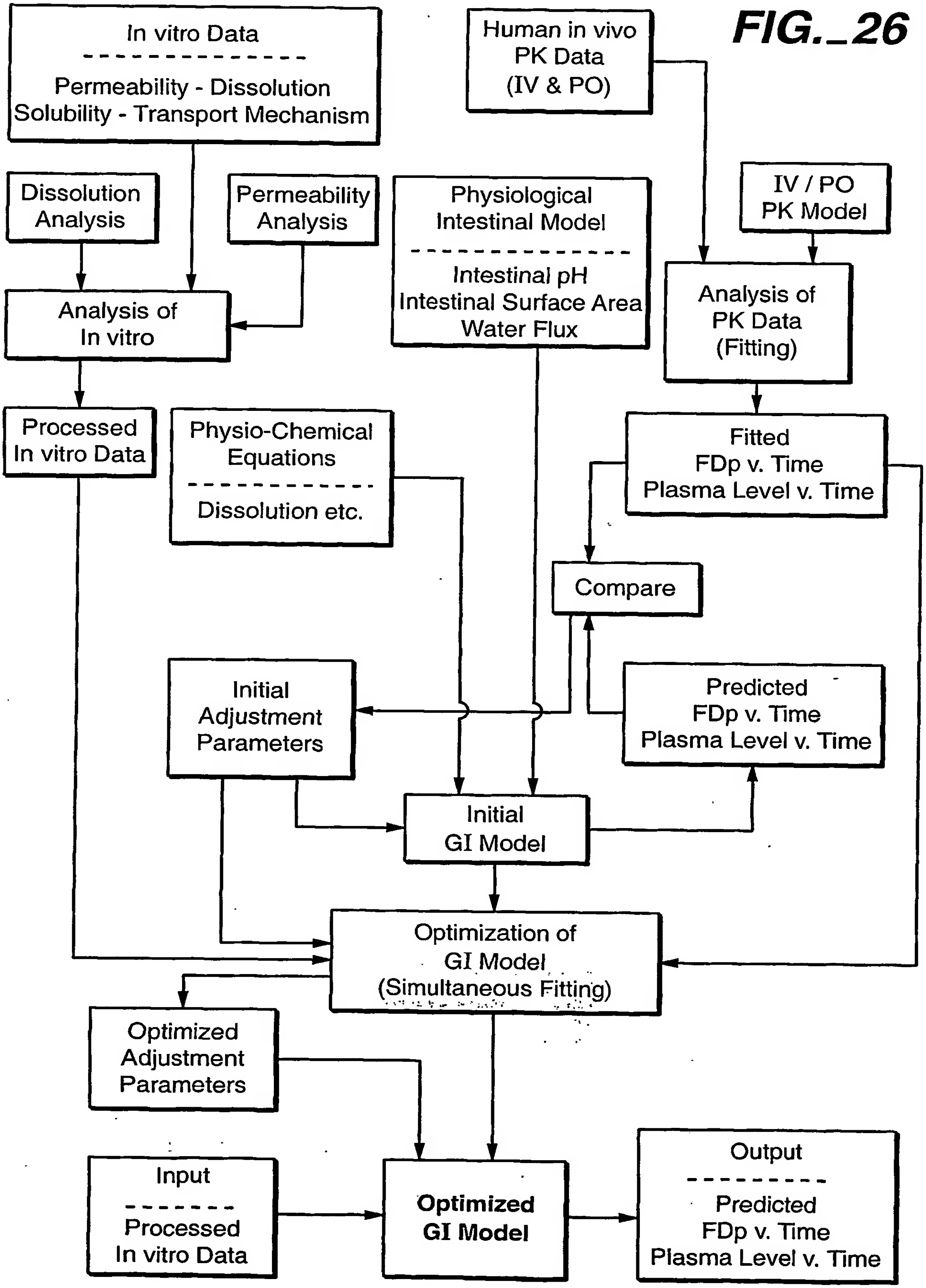


FIG._25-A
FIG._25-B

FIG._25

FIG._26


In vitro Data
Permeability - Dissolution
Solubility - Transport Mechanism
Human in vivo
PK Data
(IV & PO)
Dissolution
Analysis
Permeability
Analysis
Physiological
Intestinal Model
Intestinal pH
Intestinal Surface Area
Water Flux
IV / PO
PK Model
Analysis of
In vitro
Analysis of
PK Data
(Fitting)
Processed
In vitro Data
Physio-Chemical
Equations
Dissolution etc.
Fitted
FDp v. Time
Plasma Level v. Time
Compare
Initial
Adjustment
Parameters
Predicted
FDp v. Time
Plasma Level v. Time
Initial
GI Model
Optimization of
GI Model
(Simultaneous Fitting)
Optimized
Adjustment
Parameters
Input
Processed
In vitro Data
Optimized
GI Model
Output
Predicted
FDp v. Time
Plasma Level v. Time

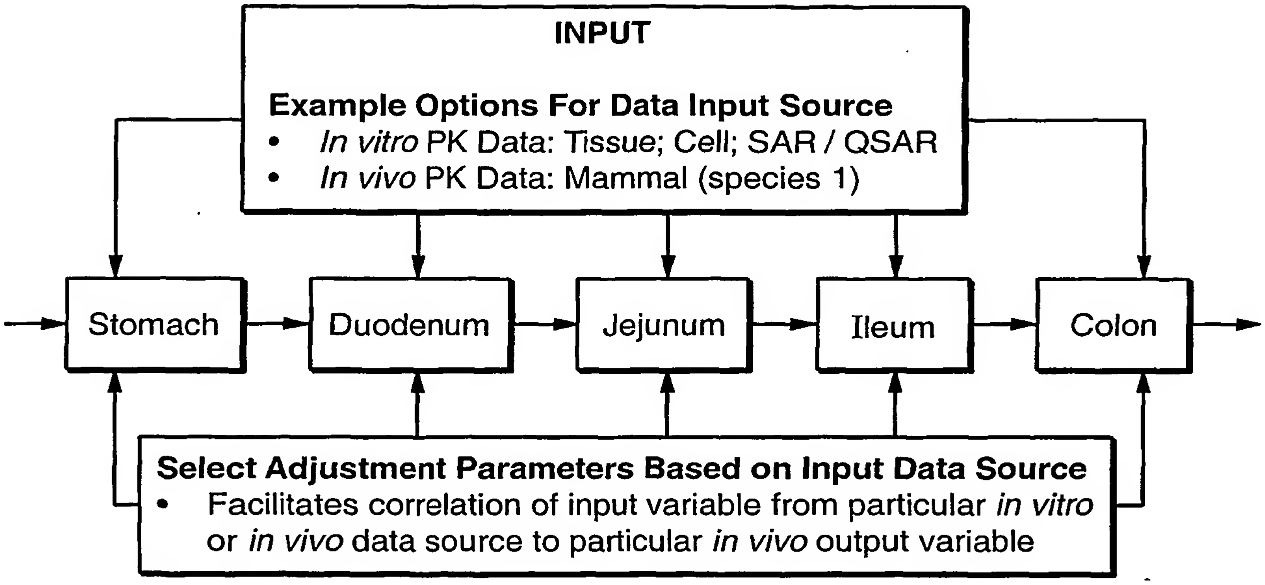

INPUT
Example Options For Data Input Source
• In vitro PK Data: Tissue; Cell; SAR / QSAR
• In vivo PK Data: Mammal (species 1)
Stomach
Duodenum
Jejunum
Ileum
Colon
Select Adjustment Parameters Based on Input Data Source
• Facilitates correlation of input variable from particular in vitro or in vivo data source to particular in vivo output variable

FIG._27

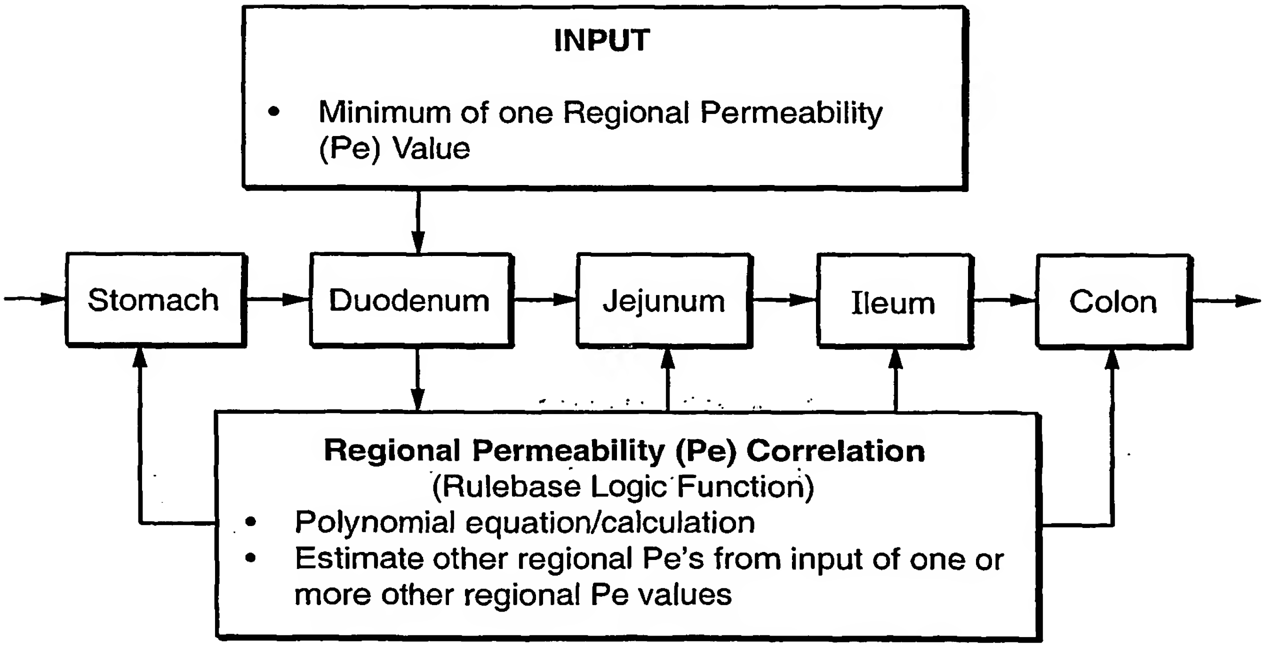

INPUT
• Minimum of one Regional Permeability (Pe) Value
Stomach
Duodenum
Jejunum
Ileum
Colon
Regional Permeability (Pe) Correlation
(Rulebase Logic Function)
• Polynomial equation/calculation
• Estimate other regional Pe's from input of one or more other regional Pe values

FIG._28

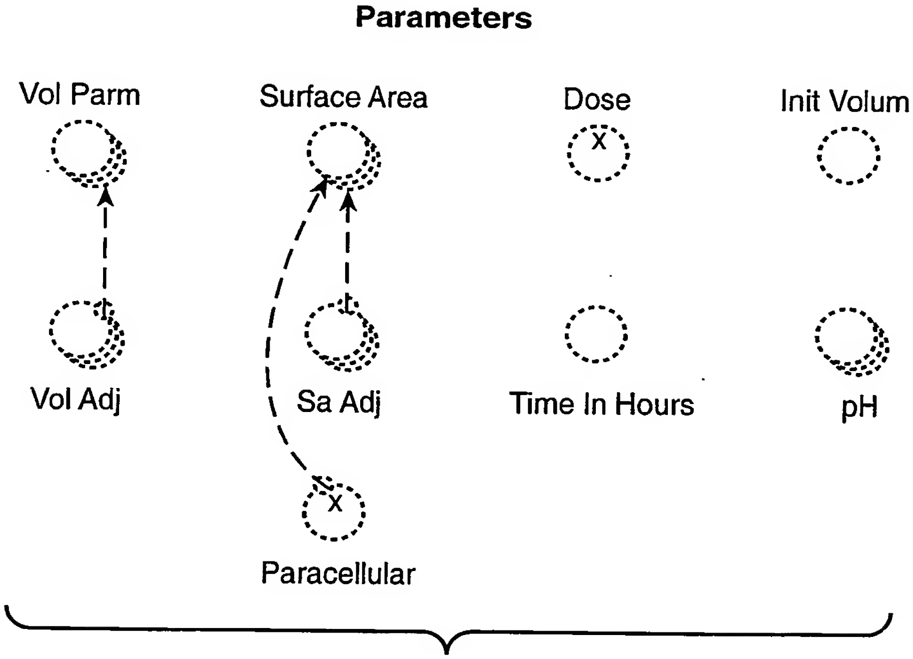

Parameters
Vol Parm
Surface Area
Dose
Init Volum
Vol Adj
Sa Adj
Time In Hours
pH
Paracellular

FIG._29

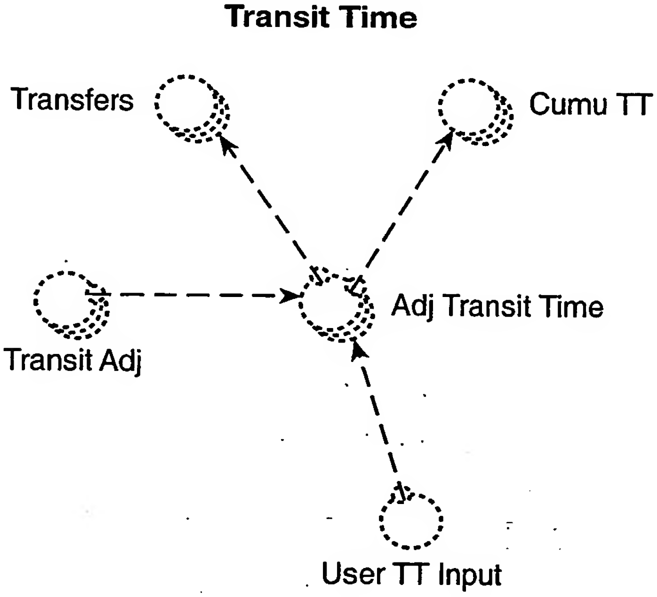

Transit Time
Transfers
Cumu TT
Transit Adj
Adj Transit Time
User TT Input

FIG._30

Permeability Calculation

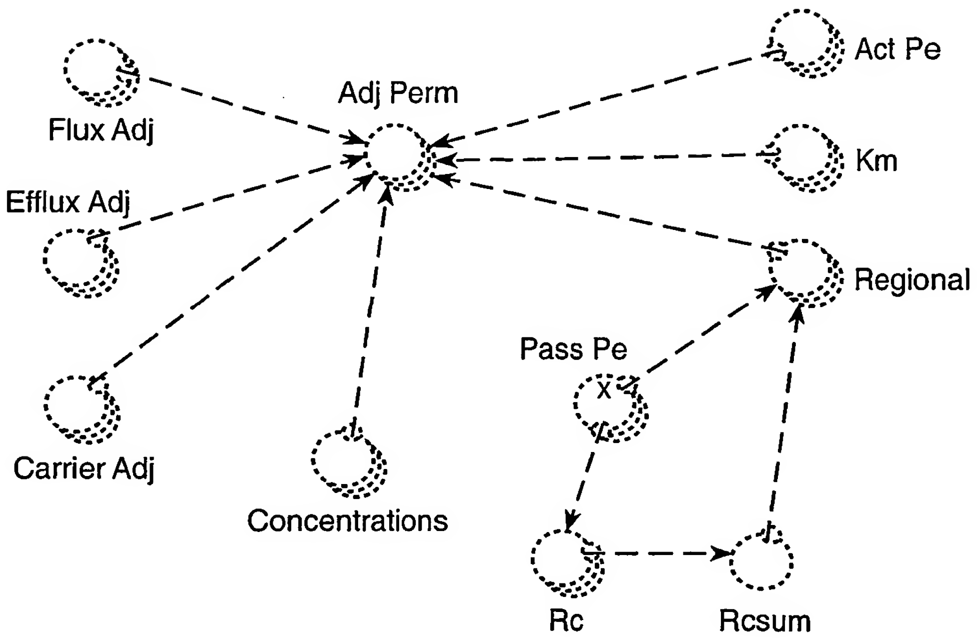

Flux Adj
Adj Perm
Act Pe
Km
Efflux Adj
Regional
Pass Pe
X
Carrier Adj
Concentrations
Rc
Rcsum

FIG._31

Solubility Calculation

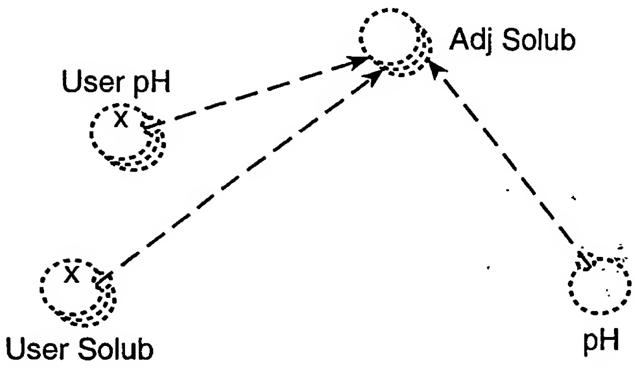

Adj Solub
User pH
X
X
User Solub
pH

FIG._32

Control Release Calculation

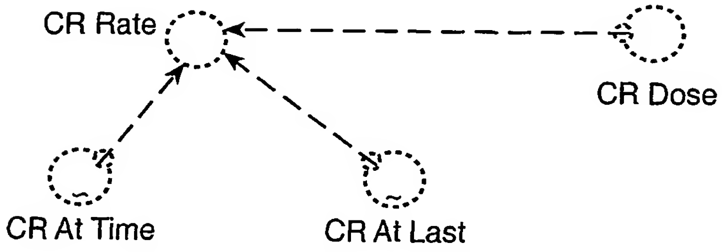

CR Rate
CR Dose
CR At Time
CR At Last

FIG._33

Concentration Calculation

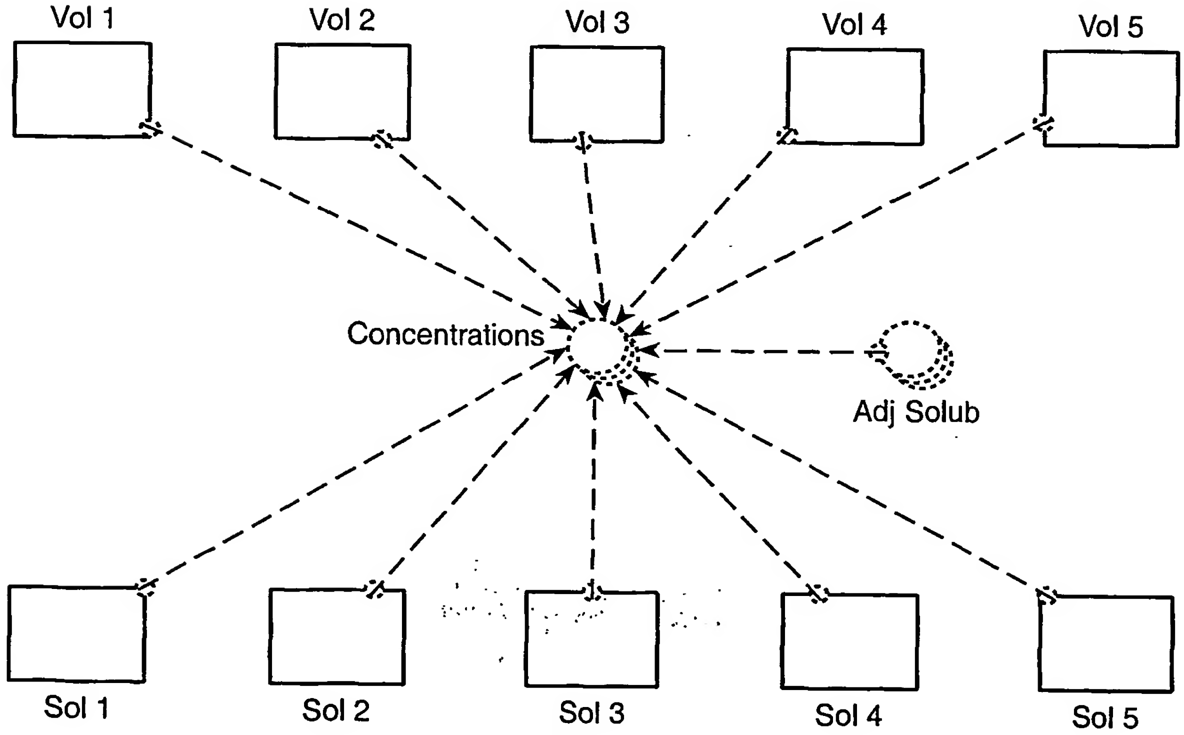

Vol 1
Vol 2
Vol 3
Vol 4
Vol 5
Concentrations
Adj Solub
Sol 1
Sol 2
Sol 3
Sol 4
Sol 5

FIG._34

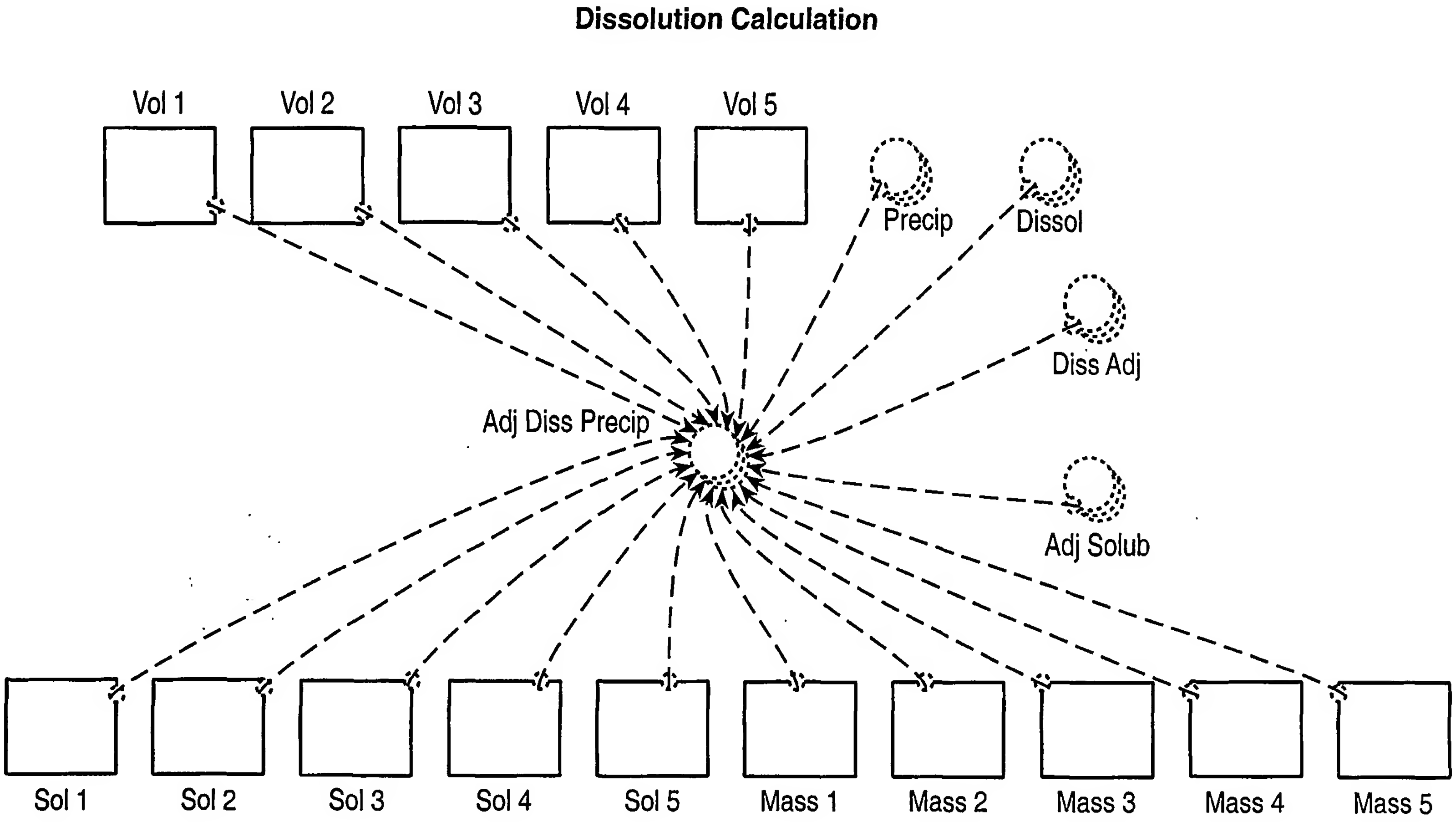

Dissolution Calculation
Vol 1
Vol 2
Vol 3
Vol 4
Vol 5
Precip
Dissol
Diss Adj
Adj Solub
Adj Diss Precip
Sol 1
Sol 2
Sol 3
Sol 4
Sol 5
Mass 1
Mass 2
Mass 3
Mass 4
Mass 5

FIG._35

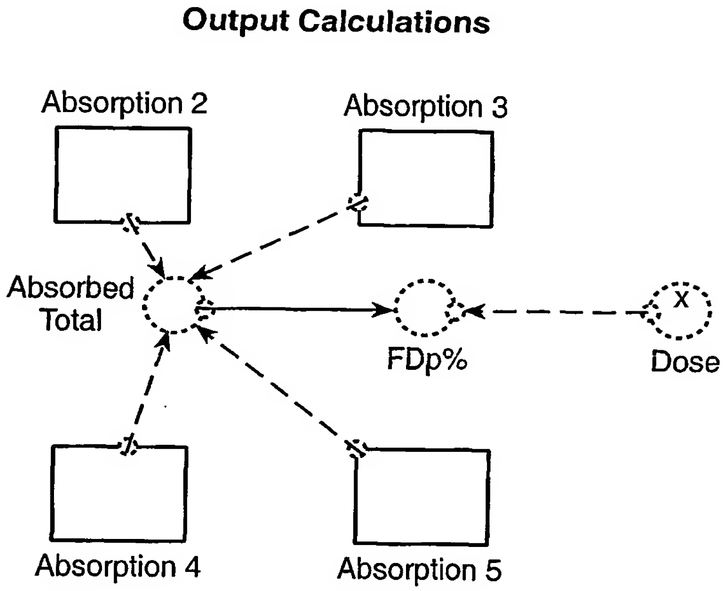

Output Calculations
Absorption 2
Absorption 3
Absorbed Total
FDp%
X
Dose
Absorption 4
Absorption 5

FIG._36

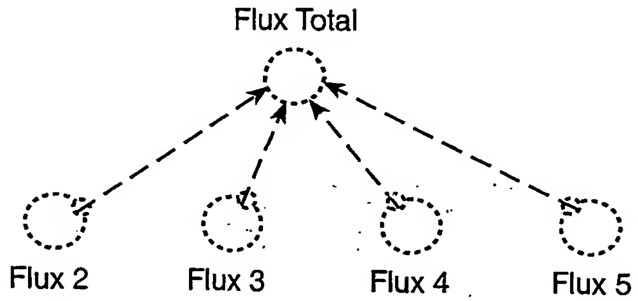

Flux Total
Flux 2
Flux 3
Flux 4
Flux 5

FIG._37

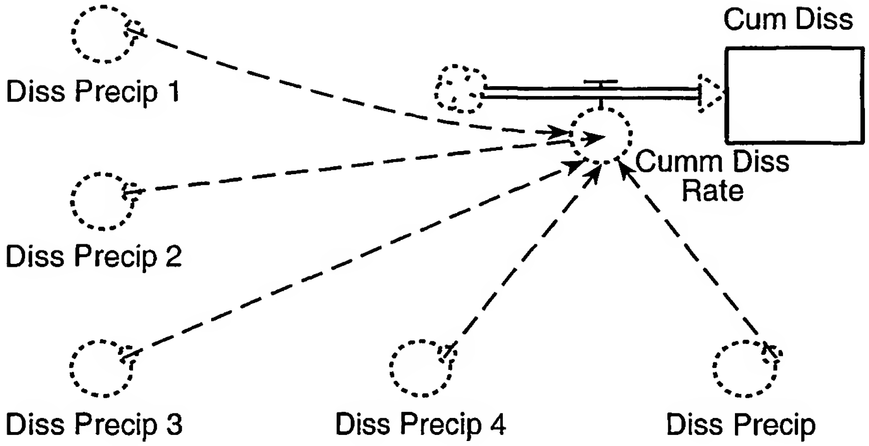

Cum Diss
Diss Precip 1
Cumm Diss Rate
Diss Precip 2
Diss Precip 3
Diss Precip 4
Diss Precip

FIG._38

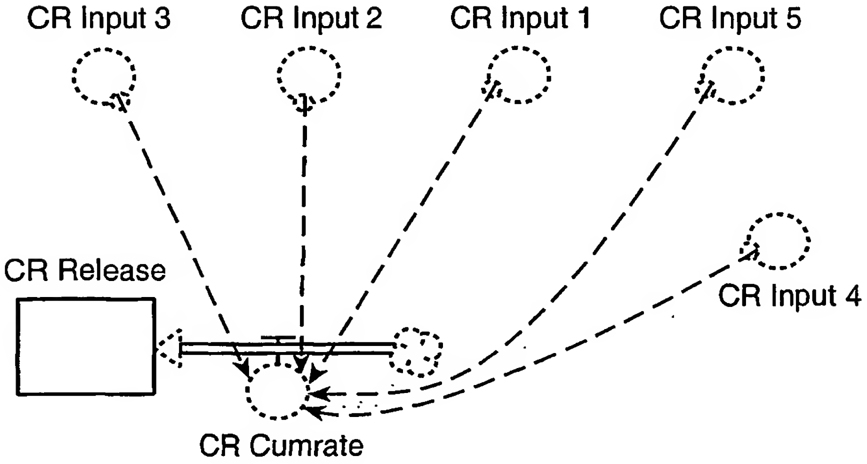

CR Input 3
CR Input 2
CR Input 1
CR Input 5
CR Release
CR Input 4
CR Cumrate

FIG._39

Physiological GI Tract Model

Database

- **GI Segment Compartments**
  - Fluid Absorption
  - Fluid Volume
  - Insoluble Mass
  - Soluble Mass
  - Soluble Mass Absorption
  - Dosage Form Mass

- **GI Segment Flow Regulators**
  - Fluid Absorption Rate
  - Fluid Volume Transit Rate
  - Insoluble Mass Transit Rate
  - Insoluble Mass Dissolution Rate
  - Soluble Mass Transit Rate
  - Soluble Mass Absorption Rate
  - Dosage Form Disintegration / Release Rate

- **GI Segment Converters**
  - Fluid Volume Absorption Rate Constant
  - GI Transit Rate Constant
  - Adjusted Dissolution Rate Constant
  - Dissolved Drug Concentration
  - Adjusted Surface Area
  - Adjusted Permeability

Rulebase

- GI Transit
- Dissolution
- Absorption
- Permeability Calculations
- Concentration Calculations
- Computational Error Corrections

FIG._40

Physiological GI Tract Model

- **GI Segment Compartments & *Flow Regulators***
    - **Fluid Absorption**
        - *Fluid Absorption Rate*
    - **Fluid Volume**
        - *Fluid Volume Absorption Rate*
        - *Fluid Volume Transit Rate*
    - **Insoluble Mass**
        - *Insoluble Mass Transit Rate*
        - *Insoluble Mass Dissolution Rate*
    - **Soluble Mass**
        - *Insoluble Mass Dissolution Rate*
        - *Soluble Mass Transit Rate*
        - *Soluble Mass Absorption Rate*
    - **Soluble Mass Absorption**
        - *Soluble Mass Absorption Rate*

FIG._41

Physiological GI Tract Model

- **GI Segments Flow Regulators & *Converters***
    - **Fluid Absorption Rate**
        - *Fluid Volume*
        - *Fluid Volume Absorption Rate Constant*
    - **Fluid Volume Transit Rate**
        - *Fluid Volume*
        - *Fluid Volume Transit Rate Constant*
    - **Insoluble Mass Transit Rate**
        - *Insoluble Mass*
        - *Insoluble Mass Transit Rate Constant*
    - **Insoluble Mass Dissolution Rate**
        - *Insoluble Mass*
        - *Dissolution Rate Constant*
    - **Soluble Mass Transit Rate**
        - *Soluble Mass*
        - *Soluble Mass Transit Rate Constant*
    - **Soluble Mass Absorption Rate (Flux)**
        - *Surface Area*
        - *Dissolved Mass Concentration*
        - *Permeability*

FIG._42

Physiological GI Tract Model

- **Converters**
  - Permeability
    - Passive Absorption Adjustment Parameter
      - Efflux / Secretion Adjustment Parameter
      - Active Absorption Adjustment Parameter
      - Active or Carrier Mediated Absorptive Permeability
      - Km
      - Passive Permeability / Regional Correlation
        - Passive Permeability
        - Logic Function For Regional Correlation
          - Passive Permeability
          - Logic Function For Regional Correlation
      - Dissolved Mass Concentrations
  - Dissolved Mass Concentration
    - Fluid Volume
    - Solubility
      - pH
      - Solubility
  - Dissolution Rate Constant
    - Fluid Volume
    - Precipitation Rate Constant
    - Dissolution Rate Adjustment Parameter
    - Solubility
    - Insoluble Mass
    - Soluble Mass
  - Surface Area
    - Surface Area Adjustment Parameter
    - Transport Mechanism
  - Transit Rate
    - Transit Time Adjustment Parameter
    - User Adjusted Transit Time
  - Fluid Volume Absorption Rate Constant
    - Fluid Volume Adjustment Parameter

FIG._43

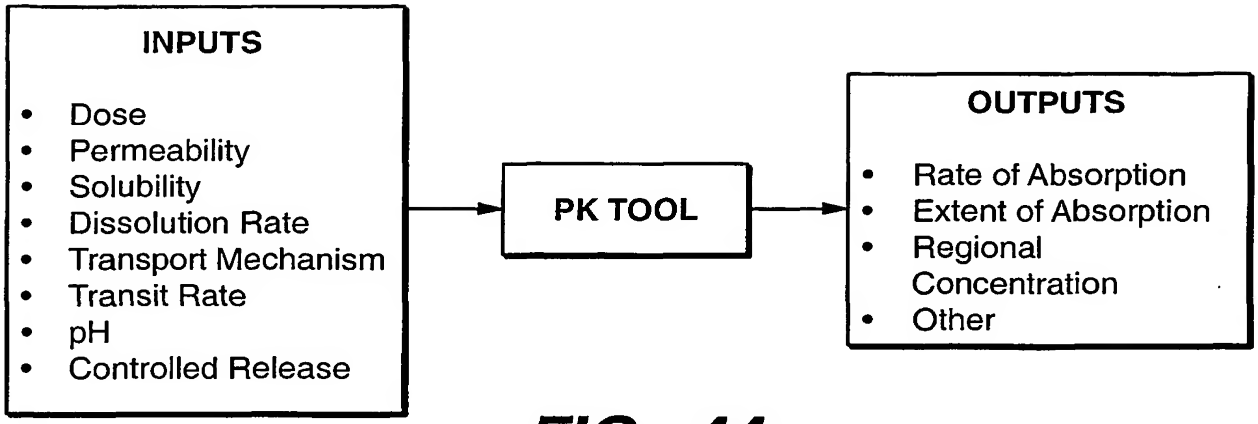

INPUTS
Dose
Permeability
Solubility
Dissolution Rate
Transport Mechanism
Transit Rate
pH
Controlled Release
PK TOOL
OUTPUTS
Rate of Absorption
Extent of Absorption
Regional Concentration
Other

FIG._44

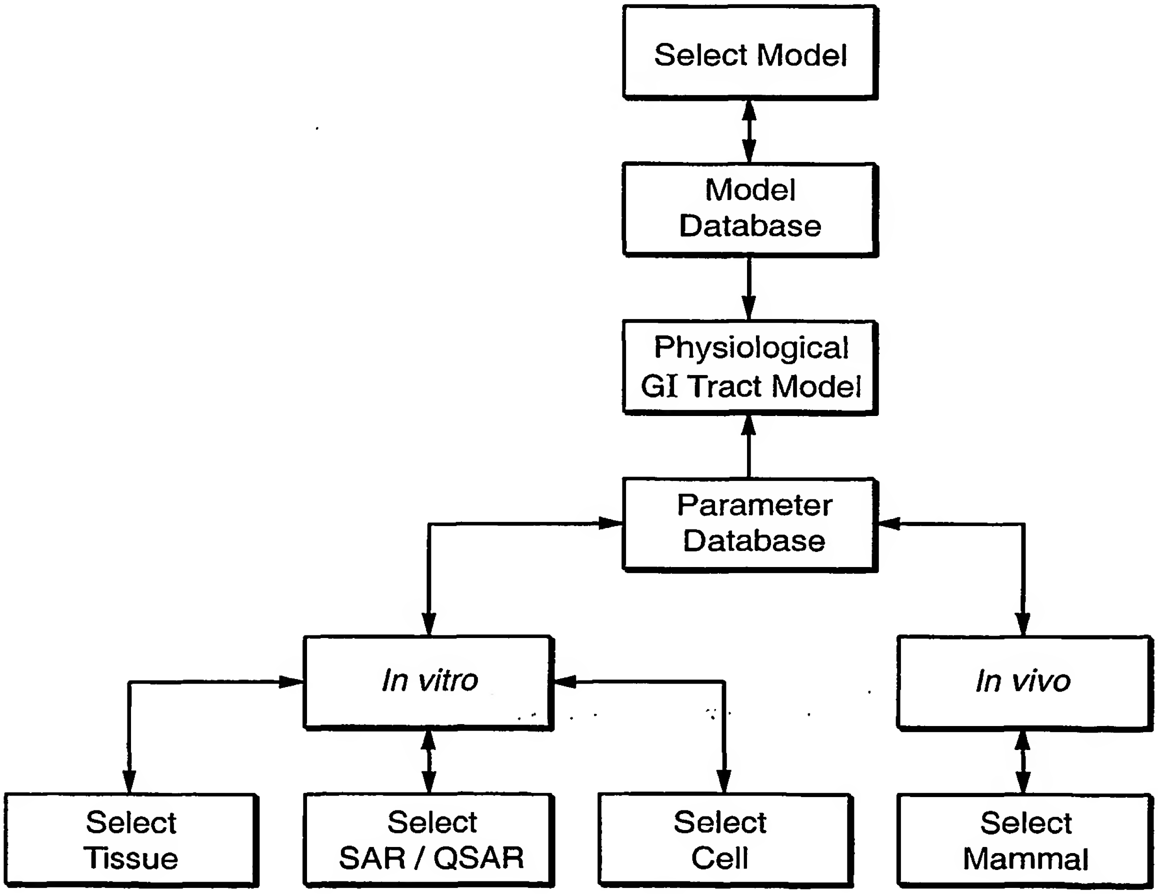

Select Model
Model Database
Physiological GI Tract Model
Parameter Database
In vitro
In vivo
Select Tissue
Select SAR / QSAR
Select Cell
Select Mammal

FIG._45

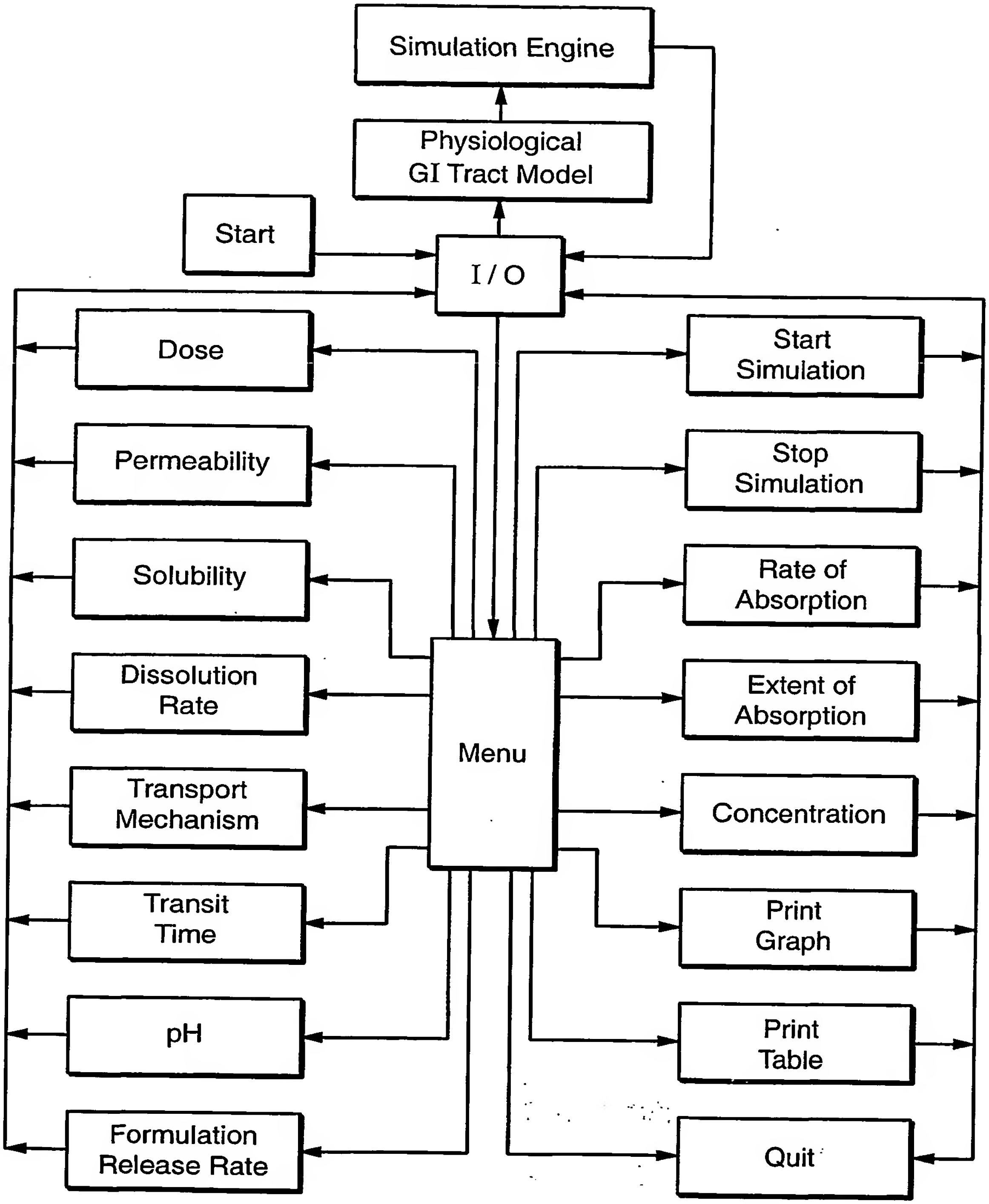

Simulation Engine
Physiological GI Tract Model
Start
I / O
Dose
Permeability
Solubility
Dissolution Rate
Transport Mechanism
Transit Time
pH
Formulation Release Rate
Menu
Start Simulation
Stop Simulation
Rate of Absorption
Extent of Absorption
Concentration
Print Graph
Print Table
Quit

FIG._46

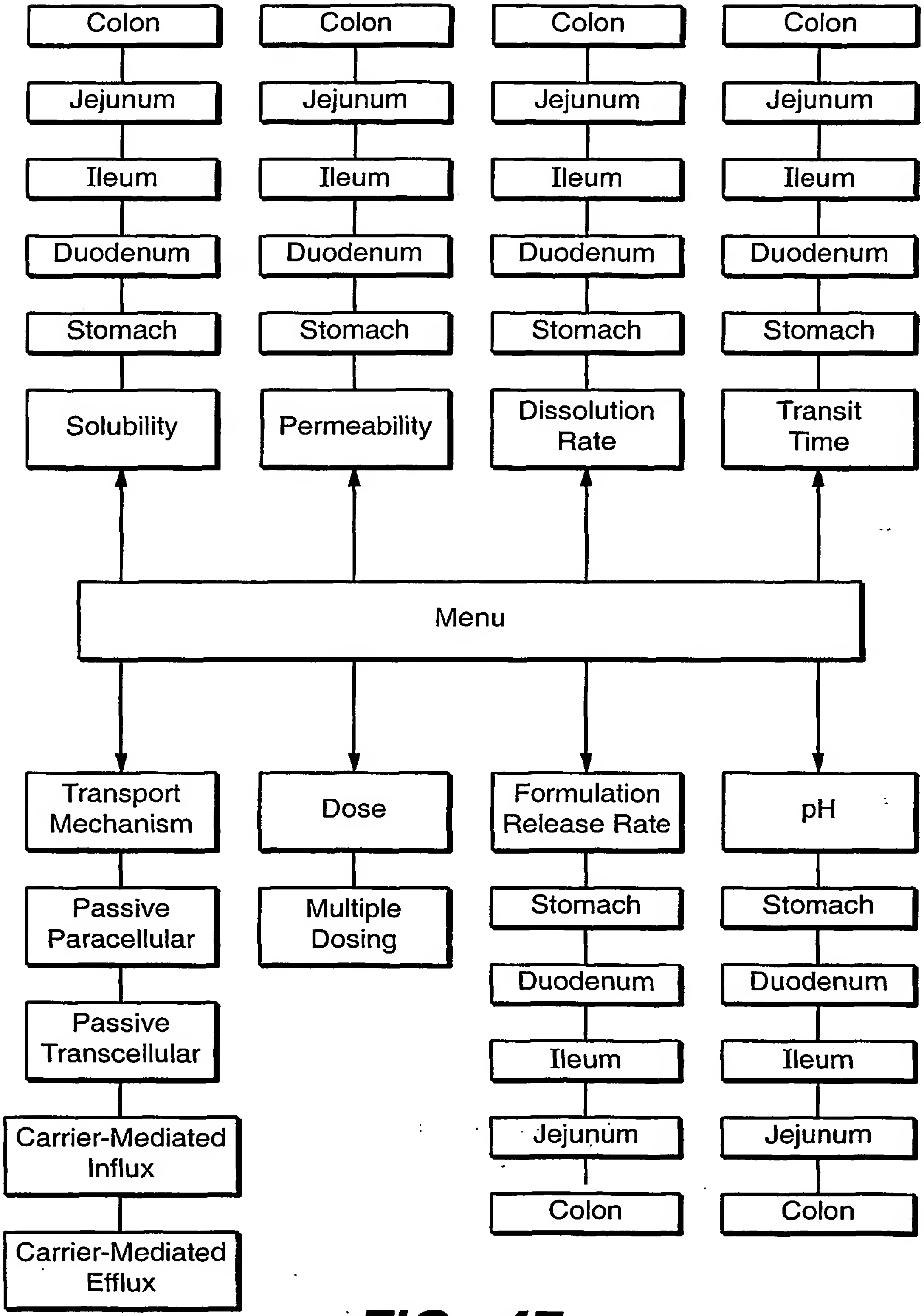

Colon
Jejunum
Ileum
Duodenum
Stomach
Solubility
Colon
Jejunum
Ileum
Duodenum
Stomach
Permeability
Colon
Jejunum
Ileum
Duodenum
Stomach
Dissolution Rate
Colon
Jejunum
Ileum
Duodenum
Stomach
Transit Time
Menu
Transport Mechanism
Passive Paracellular
Passive Transcellular
Carrier-Mediated Influx
Carrier-Mediated Efflux
Dose
Multiple Dosing
Formulation Release Rate
Stomach
Duodenum
Ileum
Jejunum
Colon
pH
Stomach
Duodenum
Ileum
Jejunum
Colon

FIG._47

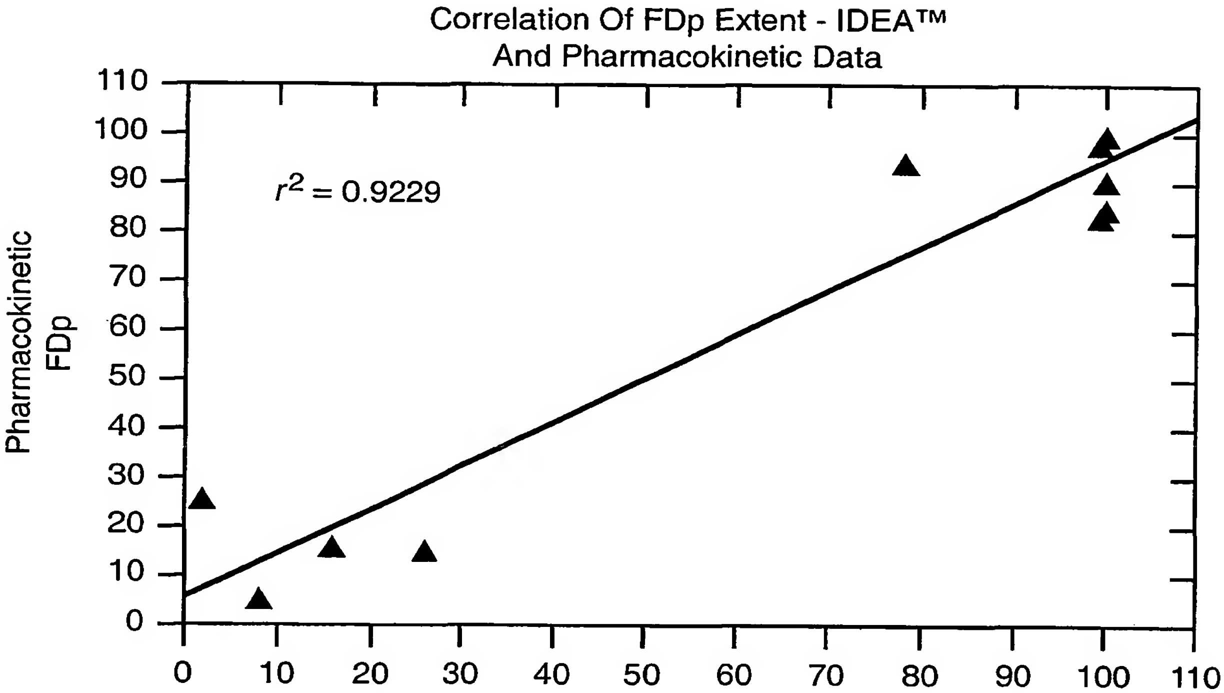

Correlation Of FDp Extent - IDEA™
And Pharmacokinetic Data
Pharmacokinetic FDp
FDp Predicted (IDEA™)
r^2 = 0.9229
0
10
20
30
40
50
60
70
80
90
100
110

FIG._48

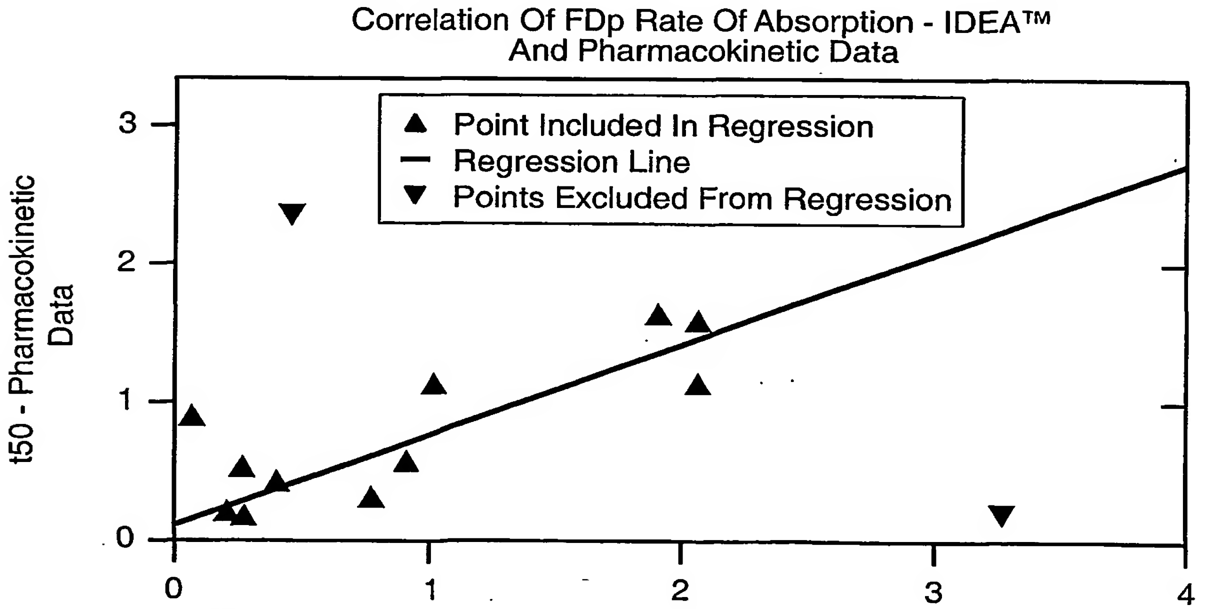

Correlation Of FDp Rate Of Absorption - IDEA™
And Pharmacokinetic Data
▲ Point Included In Regression
— Regression Line
▼ Points Excluded From Regression
t50 - Pharmacokinetic Data
t50 - IDEA™
0
1
2
3
4

FIG._49

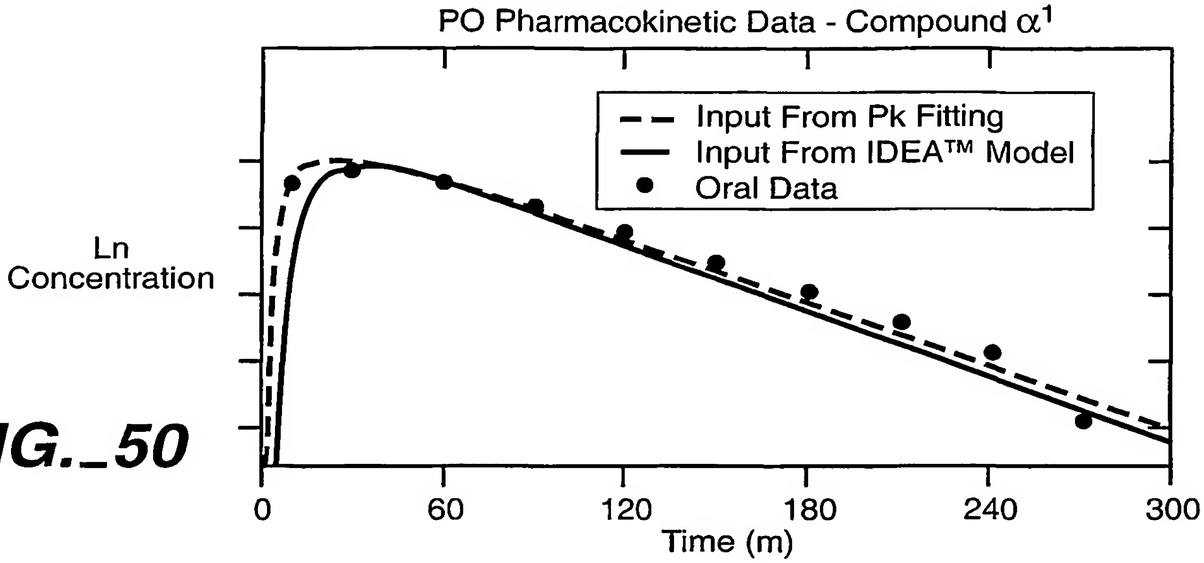


FIG._50

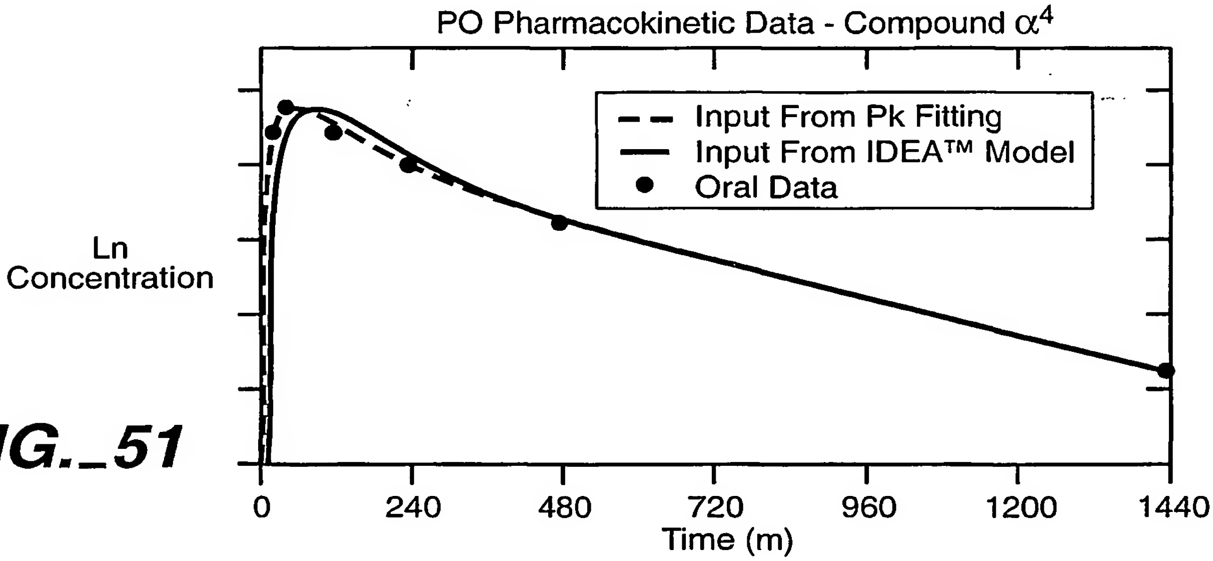


FIG._51

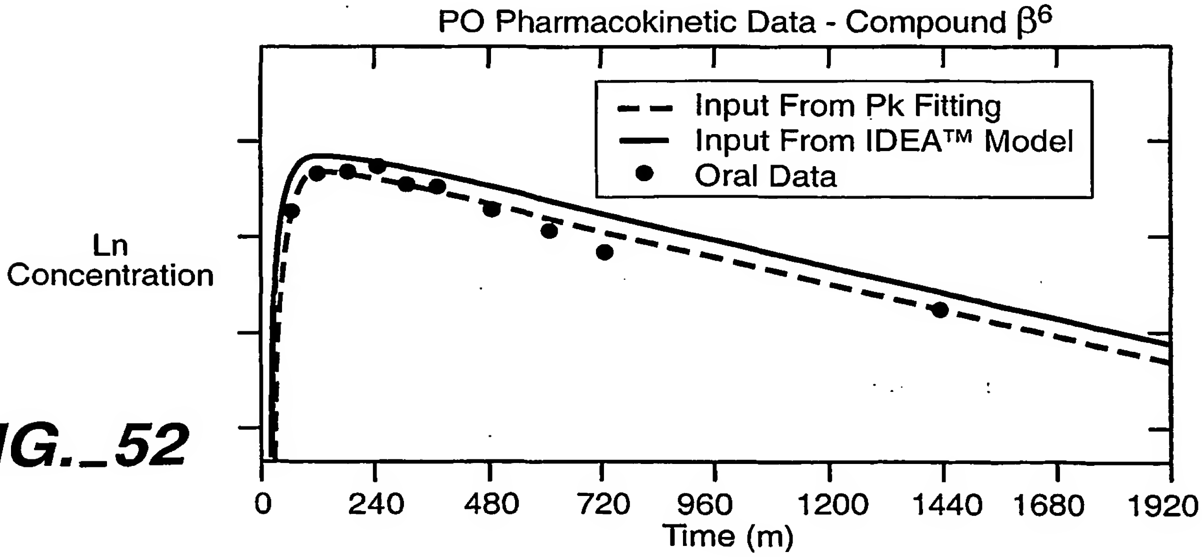


FIG._52

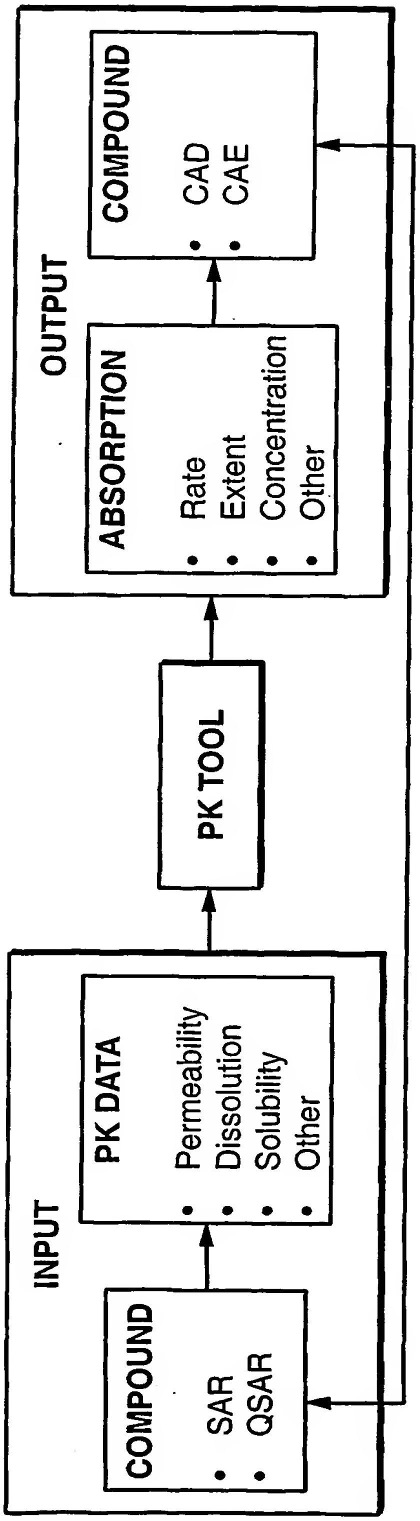

INPUT
COMPOUND
• SAR
• QSAR
PK DATA
• Permeability
• Dissolution
• Solubility
• Other
PK TOOL
OUTPUT
ABSORPTION
• Rate
• Extent
• Concentration
• Other
COMPOUND
• CAD
• CAE

FIG._53

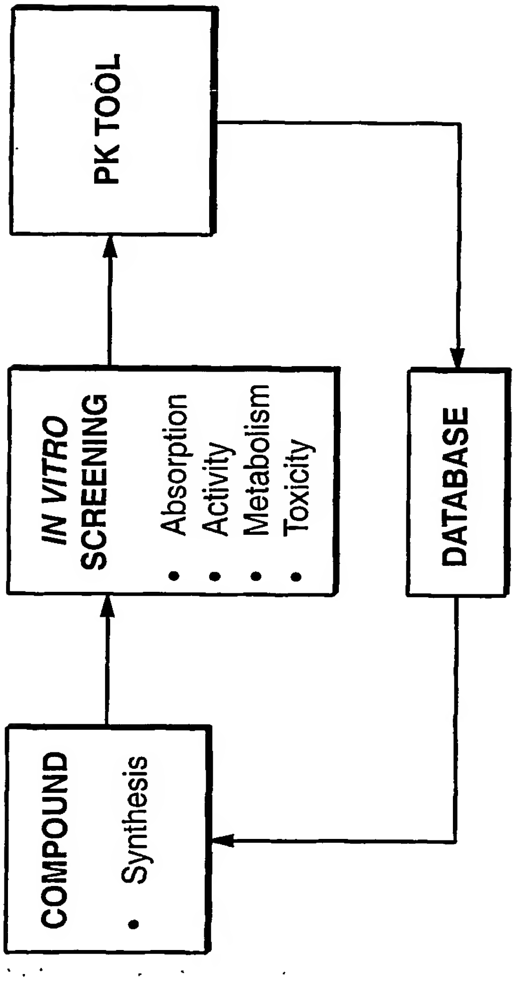

COMPOUND
• Synthesis
IN VITRO SCREENING
• Absorption
• Activity
• Metabolism
• Toxicity
PK TOOL
DATABASE

FIG._54

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/21001

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :C12Q 1/00
US CL :702/19
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 702/19

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Remington's Pharmaceutical Sciences

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Database Derwent, Accession No: 1995-263280, CARELL et al, 'Biologically active molecule combinatorial library production - by reacting core molecule with tool molecules, especially for preparation of new xanthene derivative serine protease inhibitors', abstract, WO 95/19359 A, 20 July 1995, see entire abstract.	1-199
Y	US 5,789,160 A (EATON et al) 04 August 1998, see the entire document, especially the West search report last paragraph attached to issued patent, note that Eaton et al teach among the endpoint of library screening in the SELEX system includes pharmacokinetics, bioavailability and toxicity profiles.	1-199

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance		
"E"	earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search 19 DECEMBER 1999	Date of mailing of the international search report 01 NOV 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer JOSEPH W. RICIGLIANO Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

Inte.........al application No.
PCT/US99/21001

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,770,384 A (ANDROPHY et al) 23 June 1998, see the entire document, especially the attached WEST data base search, note the reference teaches screening libraries of test compounds for pharmacokinetic parameters including bioavailability.	1-199
Y	HARVEY, S. C. Remington's Pharmaceutical Sciences. Easton, Pennsylvania: Mack Publishing Co., 1990, Chapter 35, 'Drug Absorption, Action and Disposition', pages 697-724, and Chapter 36, 'Basic Pharmacokinetics', pages 725-745, see entire document.	1-199
Y	GEX-FABRY et al. Pharmacokinetics of Drugs. Berlin: Springer-Verlag. 1994, chapter entitled, 'Considerations on data analysis using computer methods and currently available software for personal computers', pages 507-527, see entire document.	1-199
Y	Database Dialog, INSPEC Abstract Number: C82024056, HOLFORD, N.H.G. 'DRUGMODEL (pharmacokinetic modelling),' abstract, Proceedings of the fifth annual symposium on computer applications in medicinal care.' New York: IEEE, 1981, pages 603-606, see entire abstract.	1-199

Form PCT/ISA/210 (continuation of second sheet)(July 1992)★

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/21001

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest
☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/21001

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

WEST: USPAT and DERWENT FILES, DIALOG, NCBI and NLM sand NIH web sites.
Search terms included: library, combinatorial, pharmacokinetics, pharmacodynamics, gastrointestinal, absorption, bioavailability, compartments, Prophet, Drugmodel, Stella, PCNONLIN, computer, microcomputer. LANGRAN

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claims 1-53, 80-121 and 137-199, drawn to computer implemented methods/systems and computer implemented article of manufacturer and products including computer systems configured to for pharmacokinetic analysis.
Group II, claims 54-79, drawn to methods of optimizing pharmacokinetics parameters.
Group III, claims 122-126, drawn to computer subsystems for modeling the GI tract and absorption.
Group IV, claims 127-130, drawn to databases for simulating compound absorption in a mammal.
Group V, claims 131-136, drawn to computer implemented model of the GI tract.

The inventions listed as Groups I-V do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:
The invention lack unit as each group of claims is drawn to methods or computer implemented products which have different steps and different requirements. Moreover, as computer implemented pharmacokinetics modeling is known in the art the claimed inventions do not share a special technical feature.

This application contains claims directed to more than one species of the generic invention within groups I-VI. These species are deemed to lack Unity of Invention because they are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for more than one species to be searched, the appropriate additional search fees must be paid. The species are as follows:
i, a mammalian system of interest.
ii, a parameter for which the differential equations are calculated.
The species listed above do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons: Each mammalian represents a species of organ system (compartment) for which pharmacokinetics parameters will be used. This is recognized by the art and as such the recitation of different systems does not correspond to a special technical feature but rather simply a technical feature. Similarly the measurement of different parameters which are recognized in the art and the calculation of differential based equations for their presence and rates of transfer is also known in the art and hence does not constitute a special technical feature.